CASEIN

and Its Industrial Applications

By

Edwin Sutermeister

and

Frederick L. Browne

American Chemical Society
Monograph Series

SECOND EDITION

BOOK DEPARTMENT

REINHOLD PUBLISHING CORPORATION

330 WEST FORTY-SECOND STREET, NEW YORK, U. S. A.

1939

Printed in the United States of America by
THE HADDON CRAFTSMEN, INC., CAMDEN, N. J.

GENERAL INTRODUCTION

American Chemical Society Series of Scientific and Technologic Monographs

By arrangement with the Interallied Conference of Pure and Applied Chemistry, which met in London and Brussels in July, 1919, the American Chemical Society was to undertake the production and publication of Scientific and Technologic Monographs on chemical subjects. At the same time it was agreed that the National Research Council, in coöperation with the American Chemical Society and American Physical Society, should undertake the production and publication of Critical Tables of Chemical and Physical Constants. The American Chemical Society and the National Research Council mutually agreed to care for these two fields of chemical development. The American Chemical Society named as Trustees, to make the necessary arrangements for the publication of the monographs, Charles L. Parsons, secretary of the society, Washington, D. C.; the late John E. Teeple, then treasurer of the society, New York; and Professor Gellert Alleman of Swarthmore College. The Trustees arranged for the publication of the A. C. S. series of (a) Scientific and (b) Technologic Monographs by the Chemical Catalog Company, Inc. (Reinhold Publishing Corporation, successors) of New York.

The Council, acting through the Committee on National Policy of the American Chemical Society, appointed editors (the present list of whom appears at the close of this introduction) to have charge of securing authors, and of considering critically the manuscripts submitted. The editors endeavor to select topics of current interest, and authors recognized as authorities in their respective fields.

The development of knowledge in all branches of science, especially in chemistry, has been so rapid during the last fifty years, and the fields covered by this development so varied that it is difficult for any individual to keep in touch with progress in branches of science outside his own specialty. In spite of the facilities for the examination of the literature given by Chemical Abstracts and by such compendia as Beilstein's Handbuch der Organischen Chemie, Richter's Lexikon, Ostwald's Lehrbuch der Allgemeinen Chemie, Abegg's and Gmelin-Kraut's Handbuch der Anorganischen Chemie, Moissan's Traité de Chimie Minérale Générale, Friend's and Mellor's Textbooks of Inorganic Chemistry and Heilbron's Dictionary of Organic Compounds, it often takes a great deal of time to coördinate the knowledge on a given

topic. Consequently when men who have spent years in the study of important subjects are willing to coördinate their knowledge and present it in concise, readable form, they perform a service of the highest value. It was with a clear recognition of the usefulness of such work that the American Chemical Society undertook to sponsor the publication of the two series of monographs.

Two distinct purposes are served by these monographs: the first, whose fulfillment probably renders to chemists in general the most important service, is to present the knowledge available upon the chosen topic in a form intelligible to those whose activities may be along a wholly different line. Many chemists fail to realize how closely their investigations may be connected with other work which on the surface appears far afield from their own. These monographs enable such men to form closer contact with work in other lines of research. The second purpose is to promote research in the branch of science covered by the monograph, by furnishing a well-digested survey of the progress already made, and by pointing out directions in which investigation needs to be extended. To facilitate the attainment of this purpose, extended references to the literature enable anyone interested to follow up the subject in more detail. If the literature is so voluminous that a complete bibliography is impracticable, a critical selection is made of those papers which are most important.

Introduction

Preparation of the first edition of this book, which appeared in 1927, was undertaken because it was felt that there was no satisfactory work on the subject and that a volume of this sort was therefore needed. At that time casein was already long established in the coated paper industry in the United States, had recently come into wide use in water-resistant glues for woodworking and in insecticide sprays, was in the pioneering stages of development in plastics, and was being used to a minor extent in a number of other industries, such as textile printing, leather dressing, and manufacture of washable calcimine. Domestic production of casein was increasing steadily and was improving in quality, largely as a result of technical advances in methods of production by batch methods; but more than half the casein consumed was imported.

Since this book first appeared, conspicuous progress has been made in both production and utilization of casein. Further improvements in batch processes of production and in drying equipment and development of continuous processes under careful technical control have made domestic casein uniformly high in quality. Domestic production has increased very greatly while importation has fallen to less than 10 per cent of the total amount of casein consumed. Although no striking new developments have occurred in the use of casein in paper coatings, glues, insecticide sprays, textile printing, leather dressing, foods and pharmaceuticals, there have been at least three spectacular new technological achievements. Casein plastic, through improvements in processing, has become firmly established in the button business. Casein paste paint, an entirely new product, has appeared and won an important place in the paint industry, while the older powder paints have also been improved and grown in volume. A new synthetic textile fiber resembling wool has been invented and has found commercial application in Europe and Japan, though its place in American economy remains to be demonstrated.

On the theoretical side there have been many important developments in both the organic and physical chemistry of casein, some of which, such as the demonstration of the heterogeneity of casein, render the subject even more abstruse than it formerly seemed to be. That, however, is more of an excuse than a reason for the characteristically empirical approach to the technology of casein, which grows more by invention than by research. Perhaps the growth of the farm chemurgic movement will soon provide the stimulus for more effective technologic research in this field. At any rate technical understanding of the industrial uses

of casein is still in an elementary stage of development, offering abundant opportunities for interesting and possibly fruitful study.

In view of the marked changes that have taken place in the past decade it is felt that a new edition of this book is needed. Although there is some rearrangement of chapters, the general plan and scope of the book remain unaltered. Nearly all the chapters have been thoroughly revised or completely rewritten, as far as practicable by the original authors. With each chapter more extensive citations to the literature, particularly to the patent literature, are included.

The junior editor, who is chiefly responsible for assembling and co-ordinating the manuscripts for this edition, is grateful to Mr. Sutermeister for the free hand allowed him in guiding the revision and to the collaborators for their whole-hearted co-operation and kindly indulgence of his whims. To the publishers, collaborators, and readers who have patiently endured a long delay in completing his task he sincerely apologizes and in extenuation points out that the task was accepted at a time when he had good reason to expect a period of freedom from other occupations that turned out instead to be a period of exceedingly urgent demand upon his time.

U. S. Forest Products Laboratory
Madison, Wisconsin
October 19, 1938

F. L. BROWNE

CONTRIBUTING AUTHORS

George H. Brother, Ph.D., Senior Chemist, U. S. Regional Soybean Industrial Products Laboratory, Urbana, Illinois.

Don Brouse, Ph.D., Engineer, U. S. Forest Products Laboratory, Madison, Wisconsin.

Frederick L. Browne, Ph.D., Senior Chemist, U. S. Forest Products Laboratory, Madison, Wisconsin.

Edwin S. Cavett, Chem.E., Chemical Engineer, 8 Thompson Road, Marblehead, Massachusetts.

Arnold O. Dahlberg, B.S., Golden State Milk Products Company, Fresno, California.

Ross Aiken Gortner, Ph.D., Professor of Agricultural Biochemistry, University of Minnesota.

Louis A. Olney, Professor of Chemistry and Dyeing, Lowell Textile School, Lowell, Massachusetts.

Henry A. Schuette, Ph.D., Professor of Chemistry, University of Wisconsin.

Edwin Sutermeister, S.B., Chief Chemist, S. D. Warren Company, Westbrook, Maine.

Albin H. Warth, Sc.D., Chemical Director, The Crown Cork and Seal Company, Baltimore, Maryland.

Contents

Chapter 1

Casein in Milk and Its Isolation

FREDERICK L. BROWNE

The casein of commerce is a by-product of the dairy industry, and is usually prepared from skim milk, less often from buttermilk. Consideration of casein, therefore, begins logically with the manner in which it occurs in milk, its relation to the other constituents of milk, the general principles of its isolation, and the changes it undergoes during isolation. For reasons that will appear, a description of the laboratory methods of isolating casein is desirable before discussing the organic and physical chemistry of casein (Chapters 2 and 3). Commercial methods of manufacturing casein are described in Chapter 4.

Practical knowledge of casein in the form of curd and cheese dates back before the dawn of written history. Casein was recognized as a constituent of milk, together with butter fat, lactose, a little extractive substance, salt, and water, by Scheele in 1780.[118] For many years casein was regarded as a chemical individual remarkable among proteins for ease of isolation in a reasonably pure state. More recent investigations, discussed in Chapter 3, prove that casein, after it has been separated from milk, is not a homogeneous substance. The product may be separated into fractions that differ seriously in many important properties; and yet when the fractions are recombined a product apparently identical with the original casein is obtained. There may be a homogeneous protein in milk that gives rise to casein after material alteration resulting from the treatments necessary for its separation, as is known to be the case with some proteins, or there may be a mixture of proteins in milk that separates as a unit, and is called casein. At any rate the term *casein*, strictly speaking, designates a product isolated from milk rather than a biochemical substance occurring in milk. The product is clearly a mixture, but it is not yet possible to say whether some of its ingredients should be regarded as foreign substances or degradation products contaminating a pure casein, whether it is one chemical substance in various stages of polymerization or aggregation, whether it is a mixture of three or four chemical individuals that will ultimately be identified and named, or whether it is a mixture of a still more complicated nature.

There are several methods of isolating casein not only from cow's

milk but from the milk of other mammals. In view of the uncertainty as to what is properly casein and what its contaminants, the products obtained must be classified both as to source and method of preparation. This book is concerned only with the casein from cow's milk. The caseins isolated by different methods are commonly designated by the name of the originator of the method, by the principal reagent used, or by a characteristic of the process. Thus among others there will be described subsequently Hammarsten's casein, rennet casein, hydrochloric acid casein, lactic acid or naturally soured casein, and grain-curd casein. Rennet casein has more distinctive characteristics than any of the others and, when freed from inorganic constituents of milk, is commonly given a name of its own, paracasein. In this book "casein" is a general term that includes paracasein; when it is desired to exclude paracasein the term "acid casein" is used. In England "casein" often means paracasein exclusively and acid casein is then called "caseinogen." Occasionally reference will be found to alpha, beta, gamma, and delta casein but there is no general usage of such designations; "beta casein," for example, has at least three meanings according to different writers.[24, 70, 146]

Casein, in common with the other proteins, is considered an amphoteric substance, capable of combining either with acids or with bases to form salts. The salts formed with bases are called caseinates, for example, calcium caseinate; the salt formed with hydrochloric acid is called casein chloride.

Condition and Environment of Casein in Milk

The principal constituents of cow's milk and their proportions by weight are: water, 87 per cent; butter fat, 3.7 per cent; casein, 3 per cent; albumin, 0.5 per cent; globulin, 0.05 per cent; lactose, 5 per cent; citric acid, 0.2 per cent; and inorganic substances determined on analysis as ash, 0.7 per cent.[5, p. 16*] The proportions vary somewhat with breed and individuality of cow, time of milking, phase in lactation, and other factors.[15] Milk is by no means a simple, homogeneous solution of its constituents but is rather a complex colloid consisting of several phases present in widely different degrees of dispersion.[115] The coarsest dispersion is that of the butter fat, which is insoluble in water and is emulsified in droplets that range from 0.1 to 22 μ ($1\ \mu = 10^{-4}$ cm.) in diameter[142] and in a microscope are readily seen to be covered with a protective envelope of a protein[128] said to resemble haptein.[99] The droplets of fat, except those of the very smallest sizes, rise slowly in response to gravity and are removed readily in cream under the moderate centrifugal force of a cream separator.

The skim milk remaining after separation of cream still contains

* The page reference is to the book or periodical indicated, not to this work.

undissolved material in the form of particles or micelles too small to be seen with a microscope and heavier than the medium instead of lighter. The dispersed micelles of skim milk yield the casein with which this book is concerned.

That the micelles yielding casein are colloidally dispersed rather than dissolved has been known since 1818.[18, 122] The larger micelles are revealed in the ultramicroscope, where they can be seen in vigorous Brownian motion.[1, 2, 16, 62, 142] They are removed by filtration through porcelain[44, 138] or by ultrafiltration through collodion or hardened gelatin,[12] and they remain behind on dialysis.[102] Addition of sodium hydroxide, however, disperses the micelles still further until they are too small to scatter light individually in the ultramicroscope[62] or to be retained by a porcelain filter.[52] A few of the largest micelles are removed in "separator slime" in the cream separator, but longer application of greater centrifugal force removes all of them.[40, 138] In the ultracentrifuge the micelles yielding casein are thrown down relatively rapidly even with centrifugal force no greater than 1500 times gravity. Measurement by the method of velocity of ultracentrifugal sedimentation[90] proves the micelles to be polydispersed with a mean diameter of 90 mμ (1 mμ = 10^{-7} cm.) and a range in diameter from 8 to 200 mμ. About 15 per cent of the material removed in the ultracentrifuge, which may be largely calcium phosphate and fat, is in micelles or droplets larger than 200 mμ. The apparent density of the micelles is 1.50 and their shape, judging from their effect on the viscosity of skim milk, is not far from spherical. The number of micelles is estimated as 3×10^{12} per cubic centimeter.[142] There are changes in the dispersity of the micelles during such processes as creaming and churning of milk.[120]

The micelles in skim milk contain casein or its precursor in combination with calcium as a calcium caseinate. In addition the micelles contain calcium and magnesium phosphates and possibly small proportions of other substances. According to a point of view of long standing, the calcium caseinate and the calcium phosphates are united in the same micelles as a "calcium caseinate and calcium phosphate complex" or "calcium phosphocaseinate"[46] which is not regarded as a stoichiometric compound but rather as a colloidal aggregate.[106,109] There is evidence, however, that the calcium caseinate and the calcium phosphate are, at least in part, independently dispersed phases because they can be separated mechanically by centrifuging[135, 138] although the separation is not complete even when the supercentrifuge is used.[105a] Calcium sucrate tends to precipitate calcium phosphate but not calcium caseinate from milk.[108] "Synthetic skim milk" (made by dispersing casein in lime water and adding phosphoric acid solution to bring the

pH back to that of milk) contains two dispersed phases that migrate with different velocities during cataphoresis.[112] For the present the extent to which calcium caseinate and calcium phosphate are united in the same micelles is an open question. It is also uncertain whether the dispersed calcium phosphate is the tricalcium compound, $Ca_3(PO_4)_2$,[109, 126, 141] the dicalcium compound, Ca_2HPO_4,[96, 136, 138] or a mixture of the two,[56] or whether the system is a metastable state of supersaturation of various phosphates[53, 57] or an unstable state tending toward equilibrium very slowly at ordinary temperatures.[35]

From 25 to 35 per cent of the calcium and 35 to 40 per cent of the inorganic phosphorus in milk are in the serum in soluble form.[5 (p. 150), 63, 138] Dialysis of skim milk in collodion against water removes part of the calcium and phosphates,[80] which are said to be recovered as tricalcium phosphate;[102] and about 20 per cent of the calcium can be removed by treating milk with base-exchange zeolites.[76] An equilibrium between the dissolved calcium and that in the micelles is suggested by the fact that dialysis against glycerol or concentrated sucrose solution removes most of the calcium.[102] Dialysis against phosphate buffer solution of pH 6.8 removes calcium phosphate and leaves a solution containing all of the milk proteins; on subjecting this solution to ultracentrifugal sedimentation it is found to contain micelles of nine different sizes of which the six largest sizes are designated respectively δ, ϵ, ζ, η, θ, and ι casein by Pedersen and by Svedberg.[153, 154]

The white turbidity of skim milk arises from the nature and condition of dispersion of the micelles; it is lacking in milk serum, in sols of sodium caseinate or casein chloride free from denatured casein,[92] and in sols of calcium caseinate made from casein freed from phosphorus by digestion with 1-per cent sodium hydroxide solution.[81] The turbidity results from the scattering of light by micelles of sufficient size and optical density which likewise makes it possible to distinguish the micelles in an ultramicroscope. The approximate constancy of the turbidity of natural and "synthetic" skim milks and the changes effected in both turbidity and ultramicroscopic visibility when reagents are added that alter the degree of dispersion of the micelles indicate a condition of equilibrium in the size distribution of the micelles in skim milk.[26, 120, 143]

The serum left after the micelles of calcium caseinate and calcium phosphate are removed from skim milk by sedimentation, filtration through porcelain, or coagulation still contains colloidally dispersed proteins, but in a much finer degree of dispersion than the micelles yielding casein. The remaining protein consists chiefly of lactalbumin[18, 125] and lactoglobulin.[38, 124] The estimates of the size of the micelles of lactalbumin by different workers vary somewhat, the largest estimate being 5 to 15 mμ[142] and the smallest less than 1 mμ.[12] The

smaller estimate may be more nearly correct because the lactalbumin is not completely retained in ultrafiltration. Sjögren and Svedberg[125] found by ultracentrifugal sedimentation that in milk lactalbumin is monodispersed, with a micellar weight not greater than 1,000; but that when it is precipitated from milk serum with concentrated ammonium sulfate, crystallized, and redispersed, it becomes polydispersed, with micellar weights ranging from 12,000 to 25,000. More recently Pedersen[153, 154] found that the lactalbumin in skim milk dialyzed against phosphate buffer of pH 6.8 consists of two proteins, α and β, of micellar weights 17,500 and 39,000 respectively, that the γ protein, lactoglobulin, has a micellar weight of 140,000, and that the six caseins, δ to ι, are all larger than the globulin. Yet when casein is separated from milk by the procedures usually followed and is then redispersed in phosphate buffer at pH 6.8 and room temperature, the micellar weight is 75,000 to 100,000, and twice as large if redispersed at 40°C.[129] There is also some evidence that lactalbumin may be convertible, at least in part, into a globulin.[95] Apparently the micellar weights of all the milk proteins can be altered greatly in response to changing conditions.

After removal of the proteins the serum of milk is a true aqueous solution containing principally lactose and inorganic salts, salts of citric acid, and traces of minor organic compounds. Analysis of the ash of milk reveals approximately:[6, p. 111]

K_2O	25.02 per cent
CaO	20.01 " "
Na_2O	10.01 " "
MgO	2.42 " "
Fe_2O_3	0.13 " "
P_2O_5	24.29 " "
Cl	14.28 " "
SO_3	3.84 " "

The ash, of course, comes partly from the inorganic salts in the milk serum and partly from decomposition of the organic compounds. About 65 per cent of the CaO and possibly some of the MgO represent the bases in the dispersed caseinates and phosphates. Both dispersed phosphates and phosphates in true solution contribute to the P_2O_5, and in addition organic phosphorus contained in the casein appears in the ash as P_2O_5. The SO_3 likewise may come partly from organic sulfur in proteins.

In addition to the inorganic constituents given above there are in milk a few parts per million of copper, zinc, manganese and iodine. Citric acid in milk amounts on the average to 0.18 per cent[5, p. 22] and urea, uric acid, creatin, and creatinin are present to the extent of a few parts per million.[5, p. 25] There may also be the enzymes protease and lipase and others of less practical significance.[5, p. 29]

Colloid Stability and Coagulation

The separation of casein from milk is conveniently described with the aid of certain concepts of colloid chemistry which it is advisable to outline briefly. For their adequate explanation reference should be made to one of the textbooks of colloid chemistry, such as Freundlich's "Kapillarchemie," Svedberg's "Colloid Chemistry," Chapter XX in Taylor's "Treatise on Physical Chemistry," and particularly a recent paper by Kruyt.[152]

Fundamentally the stability of colloidal dispersions depends upon diffusion resulting from thermal agitation of the dispersed micelles (Brownian motion) and the molecules of dispersion medium. If, however, colliding micelles stick together, aggregates build up and coagulation rather than diffusion takes place. The cohesion or non-cohesion of colliding micelles therefore determines whether the micelles remain dispersed or coagulate. In the absence of other factors cohesion ensues because the area of interface between phases, which is a seat of energy, is thereby reduced. In general one or both of two factors may interpose to prevent cohesion and favor stability, namely, an electric charge of sufficient magnitude borne by the micelles, or a sufficient degree of swelling or hydration of the micelles to minimize the interfacial energy. Dispersions are classified as suspensoid sols or as emulsoid sols according as the micelles are stabilized by electric charge only, or by both charge and hydration. Between the typical suspensoid and the typical emulsoid sols there may be all gradations according to the relative extent to which the two factors enter into the stability of the micelles. In place of the terms suspensoid and emulsoid the terms lyophobic and lyophilic respectively are sometimes used.

In colloidal dispersions of proteins both electric charge and hydration affect the stability. With many proteins hydration predominates to such an extent that the dispersions act chiefly as emulsoid sols; but with others charge and hydration are more nearly equal in importance, and the dispersions exhibit properties intermediate between those of suspensoids and emulsoids. With all proteins the electric charge varies in sign and magnitude in response to alterations in the acidity or alkalinity of the medium. Expressing acidity or alkalinity in terms of pH (negative common logarithm of the activity of hydrogen ions), on the lower or more acid side of the pH scale the micelles are positively charged and on the higher or more alkaline side they are negatively charged. At some intermediate pH most of the micelles are electrically neutral and the dispersion is least stable. The pH at which the minimum charge and stability occur is known as the isoelectric point and is an important criterion of each protein.

Coagulation of sols is effected by sufficiently decreasing the electric

charge or hydration, whichever is the predominant factor in stability, or by decreasing both charge and hydration. Distinction is drawn between rapid coagulation, in which the stabilizing factor is reduced sufficiently to permit every collision between micelles to result in their cohesion, and slow coagulation, in which only the more vigorous collisions result in cohesion. Hydration can often be reduced by heating the sol, by adding alcohol, or by adding generous amounts of soluble inorganic salts, a procedure called "salting out." Electric charge is most conveniently reduced by addition of soluble salts, which, when the micelles are not too highly hydrated, are effective in much lower concentrations than are necessary for salting out.

Reduction of electric charge by electrolytes follows the Hardy-Schulze valence rule; the ions of charge opposite to that of the micelles are effective, divalent ions being effective at lower concentrations than univalent ions and trivalent ions at still lower concentrations, while the valence of the ions of the same charge as the micelles is relatively unimportant. In reducing hydration, however, electrolytes are effective according to the position of their ions in the lyotropic series,[150] those ions that are themselves highly hydrated being effective at lower concentrations than the less hydrated ions, regardless of valence. Typical emulsoid sols, therefore, in which hydration plays the dominant role in stability, require high concentrations of electrolytes for coagulation and the effect of position in the lyotropic series predominates over the valence rule. As the emulsoid character becomes less marked, coagulation is effected at lower concentrations of electrolyte and the valence rule becomes more prominent.

When two sols are mixed each may seriously alter the stability of the other. If one sol is positively and the other negatively charged, micelles of opposite charge join together and, if there is approximate electrical equivalence, mutual coagulation may result.* If one sol predominates, however, the mixture may remain dispersed with the charge of the micelles reduced in magnitude and the sensitivity of the sol to coagulation by electrolytes increased. On the other hand many emulsoid sols are able to act as protective colloids, that is, they render suspensoid sols

* Bungenberg de Jong makes a useful distinction between ordinary coagulation in which colliding micelles make contact and cohere firmly and coacervation, in which highly hydrated micelles of opposite charge are drawn closely together by electrostatic attraction but are kept from actual contact and coalescence by their envelopes of water, the molecules of which are oriented by polarization in one direction on the positively charged micelles and in the opposite direction on the negative micelles. Thus the micelles are electrostatically compressed against an elastic cushion of water. Coacervates of this kind often separate as a second liquid phase under suitable conditions of concentration, pH, etc., and are more readily dispersed again by altering the conditions than is the case in coagulation.[151, 152]

stable in the presence of concentrations of electrolytes that would otherwise cause coagulation.

Coagulation of typical suspensoid sols yields more or less granular precipitates, but typical emulsoid sols may coagulate in the form of flocculent or gelatinous precipitates, or, under appropriate conditions, the sol merely changes to a jelly. For the formation of a jelly the necessary conditions seem to be a sufficient degree of hydration of the micelles, a sufficient concentration of micelles, and a condition of slow coagulation.

The several dispersed phases in skim milk differ greatly in colloid characteristics. The calcium phosphate is entirely suspensoid in nature and in milk serum would be unstable if the proteins were not present.[35] It must therefore be regarded as a suspensoid protected by one or more of the proteins. Both casein and lactalbumin are known to have strong protective power;[117] it is usually assumed that the calcium phosphate is protected by the calcium caseinate.[49, 79] The calcium caseinate exhibits properties intermediate between those of suspensoids and emulsoids. It is suspensoid in that it coagulates at or near its isoelectric point, it is moderately sensitive to coagulation by inorganic salts, and the coagulating effectiveness of salts is governed more by the valence of their ions than by position in the lyotropic series. On the other hand its protective power, ease of dispersion in acids and alkalies, and formation of jellies give it marked emulsoid characteristics. Lactalbumin and lactoglobulin are predominantly emulsoid in character; they remain dispersed at their isoelectric points and are coagulated by inorganic salts only at high concentrations, the effectiveness of the salts depending chiefly upon position in the lyotropic series.

Coagulation of Casein

The micelles yielding casein in skim milk can be coagulated by heating, by addition of alcohol, by addition of salts, by bringing the skim milk close to the isoelectric point of casein, by electrodialysis, or by the action of rennet. There are, however, important differences in the products obtained by these methods and only the last three are useful for laboratory or commercial preparation of casein.

Coagulation with Heat, Alcohol, or Salts

Fresh skim milk coagulates on heating for about 12 hours at 100°, 1 hour at 130°, or 3 minutes at 150°C.[9, 139] but the time varies considerably with different milks[14] and with the previous history of the sample.[7] Solutions of casein in sodium hydroxide more alkaline than pH 6.5 also coagulate on heating.[146] The importance of the subject to the evaporated milk industry has led to many studies of heat coagulation[5, p. 205] but the mechanism of the process is not yet understood. The process is

unsuitable for preparing casein because lactalbumin and lactoglobulin, in the presence of the salts in milk, also coagulate,[29, 117] interaction between lactose and casein during heating produces a brown color,[110] and the casein itself is altered chemically and its solubility reduced.[144] When coagulation takes place the dispersed calcium phosphate is released from its association with calcium caseinate.[67]

Alcohol in sufficient concentration coagulates skim milk promptly in a manner that resembles heat coagulation to a certain extent.[5, p 213] There is, however, no sudden release of phosphates as in heat coagulation. The casein appears in the coagulum as calcium caseinate but is contaminated with other proteins, is altered chemically, and its solubility is impaired as in heat coagulation.

Calcium caseinate and calcium phosphate are coagulated by saturating skim milk with sodium chloride, lactalbumin and lactoglobulin being left behind.[2] Soluble salts of calcium or other divalent or polyvalent cations are effective at much lower concentrations than is sodium chloride.[65, 78] The method of salt coagulation is used for removing casein and phosphates in preparing lactalbumin or lactoglobulin[38, 121] but is rarely used for preparing casein.

Coagulation with Acids

Fresh skim milk is nearly neutral in reaction, with pH approximately 6.6.[36, 72, 88, 123, 134] It exerts strong buffer action through a wide range of pH with a maximum at pH 5.5, so that much acid is required to decrease the pH of skim milk through this buffer range. Much of the buffer action is due to the calcium caseinate and calcium phosphate,[140] which undergo changes in composition that consume acid. The calcium phosphate is dissolved by the acid in the form of more soluble salts that pass into true solution.[19] The calcium caseinate at the same time yields its calcium to the whey as the calcium salt of the acid added, thus releasing free casein, and the electric charge and hydration of the micelles decrease. At pH 4.6 or 4.7 the micelles become isoelectric,[87, but see 32, 65, 66] that is, the electric charge becomes effectively zero. Under these conditions the dispersion is no longer stable and the casein coagulates. The theory has been advanced, however, that the coagulation results directly from the action of the calcium ions in the whey, the concentration of which has been increased by the action of the acid on the calcium caseinate and calcium phosphate.[71] The viscosity of skim milk does not change significantly until the pH reaches the point at which coagulation sets in.[130] The coagulum, when properly separated from the whey, is chiefly free casein, and for that reason some form of acid precipitation is usually used for both laboratory and commercial preparation of casein unless rennet casein or paracasein is desired.

Any common acid may be used for making acid casein provided that it is strong enough to bring skim milk to pH 4.6 and that its calcium salts are sufficiently soluble to remain in solution in the whey. Hydrochloric acid, sulfuric acid, acetic acid, or lactic acid is often chosen for the purpose. Lactic acid need not be added directly if the skim milk is allowed to sour naturally because fermentation of lactose in the milk under the action of such organisms as *Streptococcus lactis*, which occur in milk, produces lactic acid in sufficient quantity to coagulate casein. During the early stages of natural souring the pH decreases slowly and may even increase at first, although the acidity determined by titration increases from the start.[82] The process may be speeded by adding previously soured whey as a "starter," which supplies lactic acid and additional numbers of active microörganisms. The method of natural souring has been more important for commercial than for laboratory preparation of casein in the past; but in the light of recent developments concerning the inhomogeneity of casein produced by current methods a careful reconsideration of the method of natural souring seems to be in order.

Coagulation of casein actually begins somewhat before the isoelectric point is reached.[32, 33] If acidification is stopped before attaining pH 4.6 a gelatinous curd is formed that mechanically encloses whey and is very difficult to wash thoroughly. The difficulties are increased if the acid is added carelessly and without thorough agitation, because relatively unchanged calcium caseinate and phosphate are entrained and kept from contact with the acid. When the acid is added slowly with thorough stirring until pH 4.6 is attained, the curd is more granular in nature, the calcium phosphate is more completely dissolved and the casein freed from calcium, and the whey can be washed out more thoroughly.

If addition of acid is continued beyond pH 4.6, freshly coagulated casein is redispersed, but the micelles are positively instead of negatively charged. When the pH is brought back to 4.6 with alkalies, coagulation again takes place. In contact with solutions more acid than pH 2.5 or more alkaline than pH 8.5[27, 55] casein begins to undergo chemical changes fairly rapidly. Consequently the pH should be kept well within those limits at all times during its preparation. If the temperature is kept at 5°C. or less, however, hydrolysis is greatly retarded and casein can be handled safely in solutions as acid as pH 1.0 or as alkaline as pH 10.0.[21] According to Northrup,[92] whenever casein is coagulated by addition of acid some of the casein is denatured, becomes permanently insoluble, and imparts a milky turbidity to subsequent dispersions in alkalies; but if the casein is coagulated, redispersed in acid, the solution carefully filtered, and the casein again coagulated by adding alkali, a product is obtained that forms clear solutions on both sides of the isoelectric point.

Coagulation by Electrodialysis

Casein can be coagulated by electrodialysis of skim milk.[61] The skim milk is placed in the middle one of three compartments separated by membranes permeable to dissolved substances but impermeable to the micelles yielding casein. The electrodes are placed in the outer compartments and direct current passed. Electrodialysis of the dissolved salts decreases the pH to the isoelectric point, dissolves and removes calcium phosphate, removes calcium from calcium caseinate, and finally allows the casein to coagulate.

Coagulation by Rennet

The calcium caseinate and calcium phosphate in cow's milk can be coagulated by the enzyme chymase or chymosin. Chymase-like enzymes are widely distributed in both plants and animals but the only true chymase, called rennase or rennin, is obtained from the inner lining of the fourth stomach of young calves and lambs. The extract containing rennin, as well as dried preparations from it, is called rennet. Rennin is so closely associated with, and so difficult to separate from, pepsin that some have regarded them as the same enzyme acting in different regions of pH;[93] but rennin is now known to be distinct from pepsin.[131] The composition of rennin is not yet known, but it may be a proteose and is certainly of very high dispersity because it dialyzes readily through parchment and slowly through collodion.[39, 74, 131] It is astonishingly potent; several investigators have isolated rennin one part of which was capable of coagulating from 4 to 5 million parts of slightly acidified milk.[39, 74, 131] On boiling, however, rennet becomes inactive.

Rennet coagulation takes place best at approximately 40°C. and at pH 6.0 to 6.4[86, 116] in skim milk that has not previously been heated. During the process there is no change in pH[59, 88] and very little change in other physical properties[13, 31, 41, 59, 111] except viscosity, which decreases at first and then increases rapidly as curdling proceeds.[75, 106] The rate of coagulation follows roughly and within rather narrow limits the so-called law of Segelcke and Storch, *i.e.*, "at constant temperature the time required for coagulation is inversely proportional to the quantity of rennet used,"[25, 45, 58, 68, 75] although a contrary theory that the process is autocatalytic has been advanced.[10] Under optimum conditions the coagulum, in contrast to that formed by coagulation with acids, is a firm clot or jelly which subsequently contracts and exudes whey.[32, 145] At low temperatures the clot becomes soft, at high temperatures tough and stringy,[3] and at low concentrations of casein a precipitate instead of a clot may result. Outside the range of pH 5.5 to 7.0[86] and of temperature 15 to 65°C.[5, p. 229] coagulation is inhibited. The concentration of salts in milk as well as that of casein and the pH affect the rate of coagulation with rennet and the character of the resultant clot. If the milk is

boiled before treatment with rennet, coagulation is retarded or prevented unless soluble salts such as calcium salts are added.[7, 34, 77, 85]

The product of coagulation with rennet contains casein in a permanently altered form, namely, paracasein. When paracasein is redispersed it cannot be coagulated again with rennet, but when acid casein is redispersed it coagulates with rennet in the same way as skim milk.[64] The rennet coagulum from skim milk is calcium paracaseinate containing calcium phosphate, but the content of calcium and phosphate diminish progressively if the milk is allowed to sour before adding the rennet.[44] Lactalbumin and lactoglobulin are not affected by rennet[98] and remain in the whey.

Coagulation with rennet takes place in two distinct stages, conversion of calcium caseinate into calcium paracaseinate by the action of rennin, and coagulation of calcium paracaseinate by the action of soluble salts of calcium in the milk.[47] The first stage is complete only at pH 6.0 to 6.4; beyond those limits unchanged caseinate remains in the coagulum.[116]

In spite of numerous investigations the mechanism of the first stage of rennet coagulation remains obscure. One school of thought is that rennin hydrolyzes a complex of calcium caseinate and calcium phosphate into a complex of calcium paracaseinate relatively poorer in calcium phosphate and an unidentified proteose rich in calcium that remains in the whey after the subsequent coagulation.[4, 42, 48, 51, 89, 113, 119, 126, 127] The proteose thus split off is said to represent about 4 per cent of the nitrogen of the original caseinate.[105] The splitting off of the whey proteose, however, may be merely incidental and due to other enzymes[30, 91] in ordinary rennet because rennet has been prepared in such a way that it coagulates milk without splitting off a whey proteose.[101] Van Slyke and Bosworth held that rennin splits one molecule of casein having a molecular weight of 8,888 into two molecules of paracasein having a molecular weight of 4,444,[137] but their conclusion was based upon incomplete data.[98] It will be pointed out in chapter 2 that casein and paracasein seem to be identical in chemical composition and in their chemical reactions although they differ in solubility, in their titration curves with acids and bases, in the sensitivity of their sols to coagulation with electrolytes, and in certain biochemical reactions.

There is difference of opinion about the relative degree of dispersion of the micelles of calcium caseinate and those of calcium paracaseinate before coagulation sets in. Laqueur,[64] Bleyer and Seidl,[16] and Inichoff[59] regarded calcium paracaseinate as more highly dispersed than calcium caseinate; but Loevenhart[73] thought that rennin causes association of casein into less highly dispersed paracasein. Beau postulated a depolymerization followed by a polymerization through the agency of the "plastifiers" CaO and P_2O_5, for which he proposed a detailed chemical mechanism.[11]

The suspensoid characteristics of casein sols suggested that the micelles of calcium caseinate are stabilized in milk by some other substance that is cleared away by rennin.[47, 121] Alexander assumed that lactalbumin is the protective colloid thus acted upon by rennin.[1] Lactalbumin, however, is not affected by rennin[98] and the theory fails to account for the permanent alteration of casein to paracasein.[64] The latter difficulty applies also to Bang's theory that rennin merely changes the adsorption affinity of casein for calcium.[8]

The relatively recent discovery that casein is itself a mixture of substances has led to new elaborations of the theory that rennin acts on protective colloids. It is assumed that one component of casein in milk protects the other components and is acted upon by rennin in such a way as to destroy its protective power.[58, 69, 79] It has in fact been found that casein can be separated into four components of which only one is markedly sensitive to rennin[23] and that variations in ease of coagulation of milks from different cows may be caused by variations in the proportion of this sensitive fraction in their caseins.[22]

The transformation of calcium caseinate to calcium paracaseinate effected by rennin certainly results in micelles that are more markedly suspensoid and less emulsoid in behavior,[81] particularly with respect to their sensitivity to coagulation by inorganic salts. An older hypothesis that the rennin is adsorbed by the calcium caseinate[83] foreshadowed a more explicit theory of Richardson and Palmer,[97, 112] who studied the effect of rennin on the electric charge of the micelles in calcium caseinate and calcium paracaseinate sols by observing the velocity of migration during cataphoresis. Below pH 6.9 addition of active rennet to calcium caseinate sols or to calcium caseinate and calcium phosphate sols retards cataphoretic migration and reveals a decrease in electric charge on the micelles, but it does not alter the rate of migration of similar sols of calcium paracaseinate, which migrate less rapidly than the calcium caseinate to begin with. Above pH 7.0 rennet at first hastens the migration of calcium caseinate and then leaves it unaltered. Rennin itself is isoelectric at pH 6.9 to 7.0. In the region in which rennet coagulates milk the rennin is therefore positively charged and the calcium caseinate negatively charged; mutual action of the two colloids of unlike charge is to be expected, resulting in diminished charge and increased sensitivity of the one present in dominant proportions. The objection that the proportion of rennet used is a very small fraction of the amount of casein present[19] was answered by Palmer[5, p. 226] by showing that the difference in dispersity between calcium caseinate and rennin is so great that 7 or 8 thousand molecules of rennin may be available for adsorption by each micelle of calcium caseinate.

It is generally agreed that the second stage in rennin coagulation depends upon the presence of soluble salts of calcium in the milk.

Removal of dissolved calcium leaves a milk that does not coagulate with rennet[76] and addition of calcium salts accelerates the coagulation.[100, 106] Numerous theories of the mode of action of calcium salts have been advanced.[4, 8, 32, 42, 54, 88, 113, 132, 135] The effect is not peculiar to calcium salts, however, because they can be replaced by soluble salts of any divalent or polyvalent metal. The facts can be explained reasonably as a slow coagulation of the micelles of calcium paracaseinate by calcium ions. Before adding the rennet the micelles of calcium caseinate bear a negative charge strong enough to keep the dispersion stable in the presence of the concentration of calcium ions prevailing in milk, though further addition of calcium ions causes coagulation. Rennin, in changing the caseinate to paracaseinate, reduces the charge on the micelles below the critical point for the prevailing concentration of calcium ions and coagulation results.[112]

The dispersed calcium phosphate plays a part in rennin coagulation in affecting the character of the coagulum. In the absence of calcium phosphate calcium paracaseinate usually comes down as a flaky precipitate rather than a clot or jelly,[49] a fact that gave rise to the belief that calcium caseinate and calcium phosphate are united in a complex.[48] The clot formed is firmer the more calcium phosphate there is present.[20, 103, 106] The peculiar effect on rennin coagulation caused by previously heating milk depends largely on the simultaneous presence of calcium phosphate and calcium caseinate.[104, 107] Calcium phosphate, however, can be replaced with similar results by other colloidally dispersed salts of calcium or of alkaline earth metals[47] and in fact by any negatively charged suspensoid capable of being protected by calcium caseinate, such as mastic[79] or silicic acid.[105] Further, the calcium phosphate need not be present initially in a complex with the calcium caseinate because mixtures of calcium paracaseinate sol and sols of calcium phosphate stabilized by gelatin form clots, though the mixture of calcium paracaseinate and gelatin alone forms flaky precipitates.[98] It may be that the requisite for formation of a clot rather than of a flaky precipitate is merely a sufficient concentration of micelles, because clots have been obtained with calcium paracaseinate free from other colloids.[112] The effect of calcium phosphate in rennet coagulation should be borne in mind in connection with the subsequent discussion of casein plastics and casein glues.

Laboratory Preparation of Casein

The relatively recent discoveries that casein is a mixture rather than a homogeneous substance and that it becomes denatured, hydrolyzed, or otherwise altered in micellar weight much more readily than former investigators supposed, raise serious questions about the adequacy of any

of the methods so far described for preparing casein for scientific purposes. Until systematic study by the new technics reveals the effect of variation in the method of isolation on the composition of the product, no method can be described with assurance that it yields an entirely acceptable product. In the light of present knowledge all methods used so far involve uncertainties about the extent to which the product is altered by the manipulation followed. It is therefore necessary to describe a number of important methods together with the uncertainties to which they are subject. Each investigator should weigh the uncertainties for himself and select a method accordingly.

Undue effort to free casein from supposed contaminants is inadvisable until there are more reliable grounds for deciding which of the separable components can properly be considered contaminants. More importance should be attached to isolation of the casein with as little alteration as possible than to removal of the last traces of foreign substances. Reasonable uniformity in successive batches of casein is best achieved, for the present at least, by meticulous conformity to arbitrary details of the method chosen for its isolation. As a rule those methods of preparation are to be preferred in which the product is obtained with the smallest possible number of manipulations[60] and in which the pH and temperature of the solutions are kept at all times within very conservative limits.

Most laboratory methods of preparing casein prescribe that precipitation be effected at room temperature and that the product be kept at that or lower temperature during subsequent treatment and drying. Low temperatures have the advantages of minimizing the hydrolytic and denaturing effects of acids and bases, permitting a wider range in pH[21] and longer contact of casein with aqueous media, of avoiding the alteration in micellar weight caused by heat,[129] and of yielding a product of low ash content.[19] Pasteurized or otherwise heated milk should, of course, be avoided when full advantage is to be taken of procedures at low temperatures. Commercial methods, on the other hand, usually precipitate the casein from slightly warmed skim milk because a curd more readily handled on a large scale without loss is then obtained. Commercial casein is likewise dried in warm air. For these reasons commercial casein probably differs from most laboratory preparations in micellar weight and those properties that may be affected by it. In addition, commercial casein usually has a higher content of mineral constituents than laboratory preparations. For plant control and technologic experiments intended to guide industrial processes, commercial casein is ordinarily used, although laboratory batches can be made in accordance with commercial procedures if desired. The laboratory procedures described in this chapter are of interest primarily for scientific rather than technologic purposes.

Hammarsten's Casein[50 (p. 652), 114 (p. 37)]

Dilute skim milk with four times its volume of water and precipitate the casein by adding dilute acetic acid. Purify the casein by repeatedly dissolving in water with the aid of the smallest possible quantity of alkali, filtering, reprecipitating with dilute acetic acid, and washing. Finally remove traces of fat with alcohol and ether and dry *in vacuo* over a desiccating agent.

Since the Hammarsten method makes no specific recommendation for control of pH there is danger of stopping short of pH 4.6 during acidification and of redissolving in too strongly alkaline solution. The product, when carefully prepared, contains about 0.85 per cent phosphorus of which Bosworth and Van Slyke[135] attribute 0.14 per cent to calcium phosphate entrained during precipitation. The rest of the phosphorus is organic phosphorus in the casein molecule.

Robertson[114, p. 39] reports that Hammarsten's casein, made in Germany and purchased from a chemical supply house, contains a small percentage of water-soluble acid substance that he removes by trituration with water. Its presence suggests a deterioration of the casein and the advisability of using freshly prepared casein for scientific work.

Van Slyke and Bosworth's Casein[135]

Precipitate casein as in Hammarsten's method, redissolve in ammonium hydroxide, reprecipitate and redissolve at least four times. Then add an excess of ammonium hydroxide and add ammonium oxalate to precipitate the remaining traces of calcium as calcium oxalate. Filter to remove the calcium oxalate. Finally precipitate the casein with dilute hydrochloric acid solution, wash, triturate with alcohol followed by ether, and dry in a vacuum desiccator over concentrated sulfuric acid.

Van Slyke and Bosworth claimed a reduction of the phosphorus content of the casein to 0.71 per cent, which on theoretical grounds they considered the correct content of organic phosphorus in casein.* Removal of the calcium oxalate by filtration proved difficult and tedious and the method was soon displaced by the Van Slyke and Baker method.

Van Slyke and Baker's Casein[133]

Use fresh, undiluted, unheated skim milk. Through a capillary tube discharging near the bottom of the container close to a mechanical stirrer introduce a mixture of 1 part normal hydrochloric acid solution and 1 or 2 parts normal acetic acid solution. Approach the point of coagula-

* Berggren[149] found that casein precipitated from skim milk contains 0.8 per cent organic phosphorus but that, when skim milk is dialyzed, the organic phosphorus of the casein subsequently precipitated is much lower.[148] A phosphorus content as low as 0.29 per cent was obtained in casein from milk dialyzed for 36 days. Apparently the organic phosphorus in casein is very loosely bound.

tion slowly and allow the mixture to stand several hours before adding the last small portion of acid and then let it stand 2 to 4 hours after the casein is precipitated. Wash thoroughly with successive portions of water by centrifuging until a slight cloudiness in the wash water indicates that minute particles of casein are going back into suspension. Beat the casein back into suspension with the stirrer and dissolve the free casein by adding $0.1N$ sodium hydroxide solution to pH 7.0. At pH 7.0 the remaining traces of calcium and magnesium phosphates and unaltered calcium caseinate are still undissolved and in suspension. Remove the suspended material as completely as possible by centrifuging. Then dilute the solution until it contains 1 to 1.5 per cent casein and precipitate as in the first instance except that the acid solution is normal acetic acid only. Add the acid until pH 4.7 is attained. Wash the casein, suspend it in water brought to pH 4.7 with acetic acid and remove the last traces of phosphates by electrodialysis, using collodion membranes and adding a little toluene to prevent bacterial action. Finally centrifuge with water and treat in succession with hot, neutral 70- to 80-per cent alcohol, anhydrous alcohol, anhydrous ether, and petroleum ether, benzene, or toluene.

The Van Slyke and Baker method minimizes possible alterations in casein during preparation by reducing the number of times it is treated with acids or bases and limiting the degree of alkalinity to pH 7.0. It effects final precipitation at pH 4.7 and electrodialyzes to secure maximum freedom from inorganic salts. Now that casein is known to be a mixture, however, question may be raised about the suspended material, supposedly phosphates and unaltered calcium caseinate, rejected when the initial precipitate is dissolved at pH 7.0 and centrifuged. The rejected portion may consist largely of one fraction of the casein, thereby altering the relative proportions of the different fractions in the product obtained.

The extraction with hot 70- to 80-per cent alcohol is for the purpose of removing a protein described first by Osborne and Wakeman.[94] The wisdom of this rejection is likewise open to question in the present state of knowledge of the nature of casein. On the other hand hot alcohol removes a nitrogen-free substance, apparently a lipin, not removable by cold alcohol and presumably a contaminant.[43] Certainly objectionable, however, is the fact that the extraction is made hot and we now know that casein begins to change in micellar weight even at moderate temperatures.

Cohn and Hendry's Casein[28]

Cohn and Hendry take greater precautions than Van Slyke and Baker against alteration of the casein by limiting the alkalinity to pH 6.3 and by working at the temperature of a refrigerator as much as practicable.

Contact with alcohol or with hot alcohol is avoided, possible contaminants being considered less objectionable than denaturation of the casein. The question of loss of one of the fractions of casein by incomplete solution in alkali remains. The method is described as follows:

While stirring 1 liter of skim milk add 0.05*M* hydrochloric acid solution slowly through a capillary tube extending to the bottom of the container until a 5-cc. sample diluted to 50 cc. gives a color with methyl red matching that of a sodium acetate-acetic acid buffer solution of pH 4.6. Approximately 1,000 cc. of acid is required. Add 3 liters of water and let the precipitate of casein settle in a refrigerator for 12 to 24 hours. Siphon off the supernatant liquid as completely as possible, collect the precipitate on a suction funnel, and wash with cold distilled water until the washings give no test for calcium with ammonium oxalate. To remove calcium phosphate and fat remaining as contaminants, transfer the precipitate to a 2-liter beaker and add slowly through a capillary tube extending to the bottom of the beaker, and with stirring, 0.1*M* sodium hydroxide solution until pH 6.3 is reached. The end-point is determined by means of dibromo-*o*-cresolsulfonphthalein (bromocresol purple) and phosphate buffer solutions. About 100 to 150 cc. of the sodium hydroxide solution is required. Local excess of alkali must be avoided because it tends to denature the casein. The casein is now dissolved as sodium caseinate, while fat, calcium phosphate, and any unaltered calcium caseinate are undissolved. Filter through a layer of filter paper pulp 10 to 15 mm. thick, tightly packed on a suction funnel. If the filtrate is more than slightly opalescent filter again through a fresh layer of pulp. Bring the filtrate again to pH 4.6 by adding 0.05*M* hydrochloric acid solution as in the first precipitation, the necessary amount of acid solution being determined by titration of an aliquot portion, diluted five-fold, with 0.01*M* hydrochloric acid solution. About 220 to 250 cc. of 0.05*M* acid solution is required. As the precipitation progresses add the acid solution more slowly to avoid precipitation at the tip of the capillary tube. Finally add 5 liters of cold distilled water and allow the precipitate to settle in a refrigerator. Siphon off the supernatant liquid, collect the casein on a suction funnel with hardened filter paper, wash with cold water until free from chloride, and dry over calcium chloride in a vacuum desiccator. The yield is 23 to 29 grams of a colorless, coherent product that may be readily pulverized in a mortar.

Zoller's Casein[17]

Dilute fresh, unheated skim milk with distilled water in the proportion of 1 volume of skim milk to 2 volumes of water. Warm to 30°C. Add a mixture of pure 5-per cent hydrochloric acid and 5-per cent citric acid slowly beneath the surface with rapid stirring until pH 4.6 is reached.

Stir for 10 minutes longer and then separate the casein by centrifuging in a small basket centrifuge. Resuspend the casein in distilled water adjusted to pH 4.6, stir, and centrifuge again. Repeat for the third time. Then suspend the casein in distilled water and add hydrochloric acid slowly until the final pH is 3.0. After thorough solution of the casein in the hydrochloric acid is effected, precipitate with ammonium hydroxide at pH 4.6 and wash thoroughly by centrifuging as previously described. Dry with pure acetone or alcohol.

The sodium, potassium, or ammonium caseinate made from this casein is said to be water-clear, and to give no test for calcium on spectroscopic analysis of the ash. The phosphorus and sulfur contents are 0.78 and 0.808 per cent respectively.

Grain-curd Casein

The grain-curd method of manufacturing casein, described in Chapter 4, is suitable also for laboratory preparation and has much to commend it. A single precipitation of the casein by this method, followed by thorough washing with water acidified to approximately the isoelectric point of casein, yields a product of reasonably low ash content that is suitable for many experimental purposes. If still lower ash content is necessary the product may be redissolved in dilute alkali, preferably ammonium hydroxide, the solution filtered or centrifuged, and the casein precipitated and washed a second time. In redissolving in alkali the casein should be brought back into solution as completely as possible without allowing the pH to exceed 7.5.

The chief disadvantage of the grain-curd technic for laboratory preparation of casein lies in the fact that the skim milk is warmed to a temperature dangerously close to a point at which casein is known to increase in micellar weight. The heating is required primarily to give a curd firm enough for proper handling without loss in commercial operations; on a laboratory scale it should be practicable to collect and wash thoroughly the softer and finer curd that results when precipitation is effected at lower temperatures.

Rennet Casein[112]

Rennet casein for laboratory purposes may be prepared as follows: Heat fresh skim milk to 37°C. and add commercial rennet extract in the proportion of 0.12 cc. per 1,000 cc. of milk, or in sufficient quantity to coagulate in 15 to 20 minutes. Break the curd into small pieces and stir vigorously to hasten the separation of whey. Some investigators prefer to start stirring before the curd forms. When the whey has separated let stand until the curd settles, then draw off the whey and wash with water several times. The product may be dried by one of the methods described for acid casein.

Paracasein[112]

Proceed as in making rennet casein but after the curd has been washed dissolve it in sodium hydroxide solution or in ammonium hydroxide solution, acidify carefully to pH 4.6 to dissolve the calcium phosphate and release the paracasein from combination with calcium, and purify by one of the methods described for acid casein, beginning after the first precipitation of the casein.

Electrodialysis of Casein to Remove Ash

Electrodialysis to remove ash from casein was first recommended by Van Slyke and Baker. An apparatus for the purpose and its application to casein are described by Elder *et al.*[37] By this method the ash content of an acid casein was reduced from 3.69 to 0.067 per cent in 56 hours. (Presumably the ash content reported is the so-called true ash content, which is the difference between the ash content as determined and the P_2O_5 derived from the organic phosphorus of the casein.) The suspension of casein in dilute acetic acid attained a maximum temperature of 45°C. during the electrodialysis. Removal of ash by this method could probably be effected in less time if the casein to begin with is precipitated more carefully at its isoelectric point, washed, and electrodialyzed at once without previous drying. It would be highly desirable, however, to keep the temperature of the casein suspension during electrodialysis down to 25°C. by circulating cooling water over the dialysis cell. The method proved less effective with rennet casein; the ash content of one sample reported was reduced from 7.98 to 2.17 per cent.

Drying of Casein

Laboratory methods of preparing casein usually avoid the commercial procedure of drying in warm air because the product is easily altered by heat. In solution casein is affected by temperatures as low as 40°C.[129] but the maximum temperature to which moist casein can be heated without change is not yet known. Robertson[114, p. 39] thought it safe to heat casein to 40 to 50°C. after the water had been displaced by alcohol followed by ether. On the other hand prompt drying is necessary because moist casein is easily attacked by molds.

When moist casein dries, the particles coalesce in clumps and must be ground if a fine powder is desired. To avoid coalescence and hasten drying at low temperature it has been common practice to displace the water from moist casein with alcohol and the alcohol with ether. Evaporation of the ether, however, may condense moisture unless the relative humidity of the atmosphere is very low, hence displacement of the ether with petroleum ether, benzene, or toluene has been recommended. Some writers are of the opinion that contact with alcohol even at room

temperature alters casein and for that reason Cohn and Hendry advise that casein be dried *in vacuo* over calcium chloride.

If casein is to be extracted with hot alcohol to remove Osborne and Wakeman's protein[94] and Funk's lipin[43] the extraction should be performed before the casein has dried by drawing off the water as far as possible by suction or pressure between filter papers and displacing the water with alcohol before putting the product into the extractor.

Thoroughly dry casein is wet slowly by water and therefore dissolves sluggishly in aqueous solutions.[114, p.40] Solution can be effected more rapidly by first triturating the powder with a small portion of the solution and then adding the rest. The difficulty in wetting very dry casein may be responsible for the statement that it is not appreciably hygroscopic.[17, p. 789] Casein is distinctly hygroscopic, holding 7 or 8 per cent of moisture under ordinary conditions and 20 per cent or more when in equilibrium with damp air.

The specific gravity of casein lies between 1.25 and 1.31.[5, p. 53]

References

1. Alexander, J and Bullowa, J G. M, *Arch. Pediatrics*, **27**, 18 (1910) ; *Chem. News*, **101**, 193 (1910)
2. Alexander, J., *J. Am. Chem. Soc.*, **32**, 680 (1910) ; *Proc. 8th Intern. Congr. Applied Chem.*, **4**, 12 (1912)
3. Allemann, O. and Schmid, H, *Landw. Jahrb. Schweiz*, **30**, 357 (1916) ; *Expt. Sta. Rec.*, **36**, 610
4. Arthus, M. and Pages, C., *Arch. ges. Physiol. (Pflügers)*, **2**, 331 (1890) ; Arthus, M, *Compt. rend. soc. biol.*, **55**, 795 (1903)
5. Associates of Rogers, "Fundamentals of Dairy Science," New York, Reinhold Publishing Corp., 1935
6. Babcock, S. M. and Leach, A. E., "Food Inspection and Analysis," New York, John Wiley & Sons, Inc., 1920
7. Ballowitz, K., *Biochem. Z.*, **256**, 64 (1932) ; **263**, 119, 128 (1933)
8. Bang, I., *Skand. Arch. Physiol.*, **25**, 105 (1911)
9. Bardach, K., *Monatsh.*, **18**, 199 (1897)
10. Bauer, E and Herzfeld, E, *Z. physik. Chem.*, **98**, 460 (1921)
11. Beau, M., *Lait*, **12**, 618 (1932) ; **13**, 325 (1933) ; *Chimie & Industrie, Special number* 771 (March 1932) ; *Enzymforsch.*, **4**, 173 (1935)
12. Bechhold, H., *Z. physik. Chem.*, **60**, 257 (1907), see p 307
13. Bendixen, H. A., *Proc. 20th Ann. Meeting Western Div. Am. Dairy Sci. Assoc.*, **1934**, 72
14. Benton, A. G., *J. Dairy Sci.*, **12**, 481 (1929)
15. Black, A. and Voris, L., *J. Agr. Research*, **48**, 1025 (1934)
16. Bleyer, B. and Seidl, R., *Kolloid-Z.*, **30**, 117 (1922)
17. Bogue, R. H, "Theory and Application of Colloidal Behavior," Vol. II, chap. 33, New York, McGraw-Hill Book Co., 1924
18. Bouchardat, M. G., Quevenne "Du lait," Paris (1857)
19. Brigando, J., *Lait*, **13**, 657, 843, 965, 1098, 1230 (1933)
20. Brigando, J., Champ and Closson, *Lait*, **15**, 382 (1935)
21. Carpenter, D. C., *J. Biol. Chem.*, **67**, 647 (1926)
22. Cherbuliez, E., *Compt. rend. soc. phys. hist. nat. Geneve*, **50**, 156 (1933) ; *Archiv. sci. phys. nat.*, **15**, (July-Aug. 1933)

23. Cherbuliez, E. and Meyer, Fr., *Helv. Chim. Acta*, **16**, 600 (1933)
24. Cherbuliez, E. and Schneider, M. L., *Helv. Chim. Acta*, **15**, 597 (1932); *Lait*, **13**, 264 (1933)
25. Christen, C. and Virasoro, E., *Lait*, **12**, 923 (1932); *Anales asoc. quim Argentina*, **22**, 148B (1934)
26. Clark, W. M., *J. Dairy Sci.*, **10**, 195 (1927)
27. Cohn, E. J. and Berggren, R. E. L., *J. Gen. Physiol*, **7**, 45 (1924)
28. Cohn, E. J. and Hendry, J. L., "Organic Syntheses," Vol. 10, p. 16, New York, John Wiley & Sons, Inc., 1930
29. Cosmovici, N. L., *Compt. rend. soc. biol.*, **91**, 649 (1924); **92**, 20 (1925)
30. Cosmovici, N. L., *Compt. rend. soc. biol.*, **91**, 885 (1924)
31. Cosmovici, N. L., *Compt. rend. soc. biol.*, **90**, 1313 (1924)
32. Cosmovici, N. L., *Bull. soc. chim. biol.*, **7**, 124 (1925)
33. Cosmovici, N. L., *Bull. soc. chim. biol.*, **16**, 899 (1934)
34. De Dominicis, A. and La Rotonda, C., *Ann. r. scuola agr. Portici*, **17**, 1 (1922); *Ann. chim. applicata*, **16**, 295 (1926)
35. De Toni, G. M., *Kolloid-Z.*, **28**, 145 (1921)
36. Duncombe, E., *J. Dairy Sci.*, **7**, 86 (1924)
37. Elder, A. L., Easton, R. P., Pletcher, H. E. and Petersen, F. C., *Ind. Eng. Chem., Anal. Ed.*, **6**, 65 (1934); Holmes, H. N. and Elder, A. L., *J. Phys. Chem.*, **35**, 1351 (1931)
38. Emmerling, A., *Biedermann's Zentr.*, **17**, 861 (1888)
39. Fenger, F., *J. Am. Chem. Soc.*, **45**, 249 (1923)
40. Friedenthal, H., *Ber.*, **44**, 904 (1911)
41. Fuld, E., *Beitr. chem. Physiol. Path.*, **2**, 169 (1902)
42. Fuld, E., *Biochem. Z.*, **4**, 488 (1907)
43. Funk, C., *Proc. Soc. Exptl. Biol. Med.*, **20**, 421 (1923)
44. Grimer, W. and Arlart, C., *Milchw. Forsch.*, **9**, 88 (1929)
45. Grimer, W. and Krüger, M., *Milchw. Forsch.*, **2**, 457 (1925)
46. Hammarsten, O., *Upsala Läkareföreningens förhandigar*, **9**, 363 (1873-4)
47. Hammarsten, O., *Maly's Jahrber. Tierchem.*, **4**, 135 (1875)
48. Hammarsten, O., *Nova Acta Regiae Soc. Sci. Upsaliensis in Memoriam Quattuor Saec. ab Univ. Upsaliensi Peractorum* (1877)
49. Hammarsten, O., *Z. physiol. Chem.*, **22**, 103 (1896)
50. Hammarsten, O. and Hedin, S. G., "Text Book of Physiological Chemistry," 7th ed. trans. by J. A. Mandel, New York, John Wiley & Sons, Inc., 1914
51. Harden, A. and Macallum, A. B., *Biochem. J.*, **8**, 90 (1915)
52. Harris, D. F., *J. Physiol.*, **25**, 207 (1900)
53. Hastings, A. B., Murray, C. D., and Sendroy, J. Jr., *J. Biol. Chem.*, **71**, 723 (1927)
54. Häusler, H., *Klin. Wochschr.*, **13**, 380 (1934)
55. Hoffman, W. F., *J. Biol. Chem.*, **65**, 251 (1925)
56. Hoffman, W. F. and Gortner, R. A., *J. Phys. Chem.*, **29**, 769 (1923)
57. Holt, L. E., Jr., La Mer, V. K., and Chown, H. B., *J. Biol. Chem.*, **64**, 509, 567 (1925)
58. Holter, H., *Biochem. Z.*, **255**, 160 (1932)
59. Inichoff, G. S., *Biochem. Z.*, **131**, 97 (1922)
60. Jones, D. B. and Gersdorff, E. F., *J. Biol. Chem.*, **104**, 99 (1934)
61. Kato, J., *J. Soc. Chem. Ind. Japan*, **35**, Suppl. binding 158 (1933)
62. Kreidle, A. and Neumann, A., *Arch. ges. Physiol. (Pflügers)*, **123**, 528 (1908)
63. Lampitt, L. H. and Bushill, J. H., *Biochem. J.*, **27**, 711 (1933); **28**, 1305 (1934)
64. Laqueur, E., *Beitr. chem. Physiol. Path.*, **7**, 273 (1906)
65. La Rotonda, C., *Ann. chim. applicata*, **19**, 310 (1929)
66. Lebermann, F., *Biochem. Z.*, **206**, 56 (1929)

67. Leighton, A. and Deysher, E. F., *Proc. World's Dairy Congr.*, **2**, 1276 (1923)
68. Lenk, E., *Biochem. Z.*, **178**, 105 (1926)
69. Linderstrøm-Lang, K., *Compt. rend. trav. lab. Carlsberg*, **17**, No. 9 (1929)
70. Lindet, L., *Lait*, **1**, 161 (1921)
71. Lindet, L., *Compt. rend.*, **180**, 1462 (1925)
72. Lisk, H., *J. Dairy Sci.*, **7**, 74 (1924)
73. Loevenhart, A. S., *Z. physiol. Chem.*, **41**, 177 (1904)
74. Lüers, H. and Bader, J., *Biochem. Z.*, **190**, 122 (1927)
75. Lüers, H. and Diem, A., *Milchw. Forsch.*, **2**, 405 (1925)
76. Lyman, J. F., Browne, E. H., and Otting, H. E., *Ind. Eng. Chem.*, **25**, 1297 (1933)
77. Marui, S., *Biochem. Z.*, **173**, 363 (1926)
78. Marui, S., *Biochem. Z.*, **173**, 371 (1926)
79. Marui, S., *Biochem. Z.*, **173**, 381 (1926)
80. Mattick, E. C. S. and Hallett, H. S., *J. Agr. Sci.*, **19**, 452 (1929)
81. Maynard, L. A., *J. Phys. Chem.*, **23**, 145 (1919)
82. Mayorov, S. N., *Z. Untersuch. Lebensm.*, **69**, 301 (1935)
83. Mellanby, J., *J. Physiol.*, **45**, 345 (1912)
84. Mellanby, J., *Proc. Physiol. Soc.* in *J. Physiol.*, **54**, 116 (1921)
85. Michaelis, L. and Marui, S., *Aichi J. Exptl. Med.* (Tokyo), **1**, 45 (1923)
86. Michaelis, L. and Mendelssohn, A., *Biochem. Z.*, **58**, 315 (1914)
87. Michaelis, L. and Pechstein, H., *Biochem. Z.*, **47**, 260 (1912)
88. Milroy, T. H., *Biochem. J.*, **9**, 215 (1915)
89. Müller, P. T., *Arch. Hyg.*, **44**, 144 (1902)
90. Nichols, J. B., Bailey, E. D., Holm, G. E, Greenbank, G. R. and Deysher, E. F., *J. Phys. Chem.*, **35**, 1303 (1931); *Lait*, **12**, 748 (1932)
91. Nishimura, S., *J. Agr. Chem. Soc. Japan*, **8**, 1323 (1932)
92. Northrup, J. H., *J. Gen. Physiol.*, **5**, 749 (1928)
93. Oppenheimer, C., "Die Fermente und Ihre Wirkungen," Vol. 2, p. 978, Georg Thieme, Berlin, 1926
94. Osborne, T. B. and Wakeman, A. J., *J. Biol. Chem.*, **33**, 7, 243 (1918)
95. Palmer, A. H., *J. Biol. Chem.*, **104**, 359 (1934)
96. Palmer, L. S., *Proc. Soc. Exptl. Biol. Med.*, **19**, 137 (1921)
97. Palmer, L. S., *Ind. Eng. Chem.*, **22**, 39 (1930)
98. Palmer, L. S. and Richardson, G. A., "Colloid Symposium Monograph," **3**, 112, New York, Reinhold Publishing Corp., 1925
99. Palmer, L. S. and Wiese, H. F., *J. Dairy Sci.*, **16**, 41 (1933); **17**, 29 (1934)
100. Parisi, P., *Giorn. chim. ind. applicata*, **15**, 545 (1933); *Chem. Abstracts*, **28**, 2072 (1934)
101. Petry, E., *Beitr. chem. Physiol. Path.*, **8**, 339 (1906)
102. Piettre, M., *Compt. rend.*, **193**, 1041 (1931)
103. Porcher, Ch., *Compt. rend.*, **180**, 1534 (1925)
104. Porcher, Ch., *Compt. rend.*, **182**, 1247, 1420 (1926)
105. Porcher, Ch., *Chimie & industrie*, **19**, 589, 809 (1928)
105a. Porcher, Ch., "Le Lait au Point de Vue Colloidal," p. 82, Lyons, 1929
106. Porcher, Ch., *Lait*, **10**, 667, 794, 900, 1011, 1123 (1930); **11**, 1 (1931)
107. Powell, M. E. and Palmer, L. S., *J. Dairy Sci.*, **18**, 401 (1935)
108. Pyne, G. T., *J. Agr. Sci.*, **19**, 463 (1929)
109. Pyne, G. T., *Biochem. J.*, **28**, 940 (1934)
110. Ramsey, R. J., Tracy, P. H., and Ruehe, H. A., *J. Dairy Sci.*, **16**, 17 (1933)
111. Reichel, H. and Spiro, K., *Beitr. chem. Physiol. Path.*, **8**, 15 (1906)
112. Richardson, G. A. and Palmer, L. S., *J. Phys. Chem.*, **33**, 557 (1929)
113. Ringer, S., *J. Physiol.*, **11**, 464 (1890)
114. Robertson, T. B., "Physical Chemistry of the Proteins," New York, Longmans, Green & Co., 1918

115. Röder, H., *Molkerei-Z.*, **48**, 342; *Chimie & industrie*, **31**, 1444 (1934)
116. Rona, P. and Gabbe, E., *Biochem. Z.*, **134**, 39 (1922)
117. Rupp, P., *Bull. 166, Bur. An. Ind. U. S. Dept. Agr.* (1913)
118. Scheele, C. W., *Kongl. Vetenskape Akademiens nya handlingar*, **1**, 116, 269 (1780)
119. Schmidt-Nielson, S. and Schmidt-Nielson, S., *Z. physiol. Chem.*, **60**, 426 (1909); **68**, 317 (1910); **69**, 547 (1910)
120. Schneck, A., *Milchw. Forsch.*, **7**, 1 (1928-9)
121. Schryver, S. B., *Proc. Roy. Soc.* (London), **B86**, 460 (1913)
122. Schübler, *Deut. Archiv. Physiol.*, No. 4 (1818) cited by Kreidle and Neumann, *Arch. ges. Physiol. (Pflügers)*, **123**, 528 (1908)
123. Schultz, E. W., Marx, A., and Beavers, H. J., *J. Dairy Sci.*, **5**, 383 (1922)
124. Sebelien, J., *Z. physiol. Chem.* **9**, 445 (1885)
125. Sjögren, B. and Svedberg, T., *J. Am. Chem. Soc.*, **52**, 3650 (1930)
126. Söldner, F., *Landw. Vers.-Sta.*, **35**, 351 (1888)
127. Spiro, K., *Beitr. chem. Physiol. Path.*, **8**, 365 (1906)
128. Storch, V., *Analyst*, **22**, 197 (1897)
129. Svedberg, T., Carpenter, L. M., and Carpenter, D. C., *J. Am. Chem. Soc.*, **52**, 241, 701 (1930); **53**, 1812 (1931)
130. Tapernoux, A. and Vuillaume, T., *Lait*, **14**, 449 (1934)
131. Tauber, H. and Kleiner, I. S., *J. Biol. Chem.*, **96**, 745, 755 (1932); **104**, 259 (1934)
132. Van Dam, W., *Z. physiol. Chem.*, **58**, 295 (1908)
133. Van Slyke, L. L. and Baker, J. C., *J. Biol. Chem.*, **35**, 127 (1918); Van Slyke, *Proc. World's Dairy Congress*, **2**, 1145 (1923)
134. Van Slyke, L. L. and Baker, J. C., *J. Biol. Chem.*, **40**, 345 (1919)
135. Van Slyke, L. L. and Bosworth, A. W., *J. Biol. Chem.*, **14**, 203, 207 (1913)
136. Van Slyke, L. L. and Bosworth, A. W., *J. Biol. Chem.*, **14**, 211 (1913)
137. Van Slyke, L. L. and Bosworth, A. W., *J. Biol. Chem.*, **14**, 227 (1913); Bosworth, *J. Biol. Chem.*, **19**, 397 (1914)
138. Van Slyke, L. L. and Bosworth, A. W., *J. Biol. Chem.*, **20**, 135 (1915); *Tech. Bull. 39 N. Y. (Geneva) Agr. Expt. Sta.* (1914)
139. Webb, B. H. and Holm, G. E., *J. Dairy Sci.*, **15**, 345 (1932)
140. Whittier, E. O., *J. Biol. Chem.*, **83**, 79 (1929)
141. Whittier, E. O., *J. Biol. Chem.*, **102**, 733 (1933)
142. Wiegner, G., *Nahr. Genussm.*, **27**, 425 (1914)
143. Wiegner, G. and Yakuwa, G., *Milchw. Zentr.*, **5**, 473, 521 (1909)
144. Wright, N. C., *Biochem. J.*, **18**, 245 (1924); *J. Dairy Research*, **4**, 122 (1932)
145. Würster, K., *Milchw. Forsch.*, **16**, 200 (1934)
146. Zoller, H. F., *Science*, **52**, 614 (1920)
147. Zsigmondy, R., *Z. anal. Chem.*, **40**, 697 (1901)

Additional References

148. Berggren, R. E. L., *J. Biol. Chem.*, **95**, 451 (1932)
149. Berggren, R. E. L., *J. Biol. Chem.*, **95**, 461 (1932)
150. Heymann, E., Bleakley, H. G. and Docking, A. R., *J. Phys. Chem.*, **42**, 353 (1938)
151. Jong, H. G. B. de, "La Coacervation, les Coacervates, et leur Importance en Biologie" Nos. 397 and 398, Hermann et Cie, Paris (1936) and numerous papers in *Kolloid-Z.*, *Kolloid Beihefte*, and *Biochem. Z.*
152. Kruyt, H. R., *Bull. soc. chim.*, [5] **4**, 1925 (1937)
153. Pedersen, K. O., *Biochem., J.*, **30**, 948 (1936)
154. Svedberg, T., *Ind. Eng. Chem., Anal. Ed.*, **10**, 113 (1938) see p. 123

Chapter 2

The Organic Chemistry of Casein

Ross Aiken Gortner

Casein belongs to that group of organic compounds known as proteins. The proteins are, without doubt, the most complex organic compounds with which the chemist has to deal, and within this group casein belongs to one of the more complex subdivisions, inasmuch as it is not a simple protein but belongs to the sub-class of phosphoproteins.

Probably no single protein has been the subject of as many chemical investigations as has casein. This is to be expected, as it is the characteristic protein in the milk of all mammals and can be readily isolated in a more or less pure state. The present evidence that casein is a mixture rather than a chemical individual is reviewed in Chapter 3. Owing to its ready availability the casein of cow's milk has been investigated more often than any other.

Elementary Analysis and Molecular Weight of Casein

Typical elementary analyses of casein from cow's milk, woman's milk, and milk of other animals are shown in Table 1. It can readily be seen that caseins prepared from different sources cannot be identified by elementary analysis.

Determination of the empirical formula is extremely difficult. Cohn, Hendry and Prentiss[18] reviewed the evidence and concluded that the minimum molecular weight is 12,800 but that the actual molecular weight is probably 192,000. Whether or not they are justified in all of their assumptions, the evidence is overwhelming that the actual molecular weight is much greater than 12,800.

Svedberg, Carpenter and Carpenter[105] studied the "molecular weight" of casein by ultracentrifugal sedimentation. They found that Hammarsten's casein is a mixture of protein particles of different micellar weights. If, however, Hammarsten's casein was extracted with acid-alcohol, about 33 per cent dissolved and after purification was essentially homogeneous in solution, with a micellar weight of 375,000. Van Slyke and Baker's casein proved to be a mixture of different micellar weights with the fraction between 75,000 and 100,000 predominating. When the crude moist casein was heated to 40°C. during its dispersion in the buffer solutions all particles having micellar weights between 75,000 and

TABLE 1.—Representative Elementary Analyses of Casein from Cow's Milk and Milk of Other Mammals

Source of casein and name of analyst	Carbon	Hydrogen	Nitrogen	Sulfur	Phosphorus	Oxygen (by difference)
	Content in per cent by weight of					
Cow's milk:						
Hammarsten[50]	52.96	7.05	15.65	0.72	0.85	22.77
Lehman, Hempel[53]	54.00	7.04	15.60	0.77	0.85	21.74
Tangl[106]	52.69	6.76	15.65	0.83	0.88	23.19
Van Slyke and Bosworth[121]	53.50	7.13	15.80	0.72	0.71	22.14
Woman's milk:						
Wroblewski[129]	52.24	7.32	14.97	1.12	0.68	23.67
Ewe's milk:						
Tangl[106]	52.92	7.05	15.71	0.72	0.81	22.79
Goat's milk:						
Tangl[106]	52.90	6.86	15.48	0.70	0.76	23.30
Mare's milk:						
Tangl[106]	52.36	7.09	16.44	0.53	0.88	22.70
Ass's milk:						
Tangl[106]	52.57	7.01	16.28	0.59	1.06	22.49
Rabbit's milk:						
Burow[16]	52.87	7.05	15.60	0.73	0.81	22.94

100,000 were transformed into particles having micellar weights of the order of 188,000.

These and other studies prove that the term "molecular weight" is misapplied to casein sols, for no constant, characteristic value has yet been found. Apparently casein may be dispersed in colloidal sols, and when so dispersed it has a variable micellar weight depending upon the past history of the casein, its method of preparation, the pH, and other factors of the environment. By appropriate fractionation, preparations having a relatively constant micellar weight can be obtained, but there is no final evidence that these are individual chemical compounds as distinguished from physical aggregates.

Decomposition Products and Structure of Casein

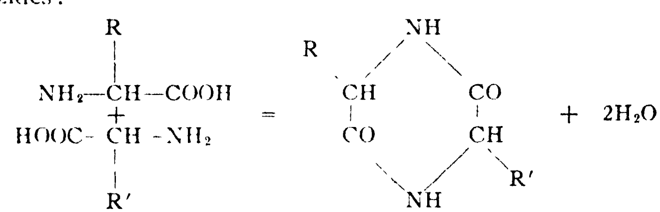
Amino Acid Content of Casein

The first decomposition product of a protein to be isolated in pure form was leucine (isobutyl α-amino acetic acid) discovered by Proust[85] in 1818 in cheese and called by him "oxide caséeux." Glycine (α-amino acetic acid) was isolated from gelatin and meat by Braconnot[13] in 1820. Liebig[69] in 1846 isolated a crystalline compound from casein that had been hydrolyzed by strong alkali. Bopp[12] and Hinterberger[55] later isolated the same compound from other proteins, using both acid and alkaline hydrolysis, and called it tyrosine (*p*-hydroxy benzene α-amino propionic acid); Cramer[10] added serine (β-hydroxy α-amino propionic acid) isolated from silk gum, Ritthausen[89] obtained aspartic acid

(α-amino succinic acid) and glutamic acid (α-amino *n*-glutaric acid) from vegetable proteins. Weyl[125] added alanine (α-amino propionic acid) obtained from silk fibroin.

These were the only amino acids isolated from proteins prior to 1892. During this earlier period many workers studied the proteins by the conventional methods of organic chemistry, oxidation, reduction, destructive distillation, nitration, halogenation, etc., apparently thinking that the protein molecule was much simpler than we now know it to be, and that the amino acids that had been isolated were secondary decomposition products. Unfortunately few of these studies yielded much information about the real nature of the proteins, and it was not until the epoch-making studies of Emil Fischer[32, 33, 34] that the true nature of the proteins began to emerge.

We now know that the amino acids are primary decomposition products of proteins and that the proteins themselves may be regarded as condensations of amino acids with the elimination of water. The exact mode of the condensation is still an open question. Fischer thought that the amino acids are united through the peptide linkage, —NH—CO—, largely because of his synthesis of polypeptides identical with polypeptides which he isolated from natural proteins. This chain-like, peptide-linked structure of proteins was recently questioned by Abderhalden,[1] who proved that some at least of the amino acids may be present in proteins as anhydride combinations in substituted diketopiperazides:



and that the diketopiperazide rings may be held together by secondary valences to form the protein molecule. How far Fischer's theory of peptide linkage must be modified to account for Abderhalden's experimental results cannot yet be determined. Possibly both structures exist in the protein molecule, although the evidence favors predominance of the simple peptide linkage.*

* Sadikov and Poshiltzova[151] believe that the molecules of proteins are made up of many rings. Besides cyclodipeptides such as Abderhalden's diketopiperazides they isolated the cyclotripeptide, cyclo-prolylprolylleucine. They consider that cyclopeptides combine with one another in two ways, first to form cyclocyclines, and second, through their enol hydroxyl groups, to form di-, tri-, and polypeptines. Bancroft and Browning,[131] as usual favoring the minority in a good controversy, consider that "the normal peptide linkage is not so prevalent as organic chemists have assumed."

Table 2.—Percentages of Amino Acids and Other Decomposition Products of Casein found by Various Investigators

Amino acid or other decomposition product	Per cent	Found by	See also note
Glycine (α-amino acetic acid)	0.45	Foreman[38]	
Alanine (α-amino propionic acid)	1.85	Foreman[38]	*a*
Valine (α-isopropyl-α-amino acetic acid)	7.93	Foreman[38]	*b*
Norvaline (α-amino-*n*-valerianic acid)	0.20	Abderhalden and Reich[3]	*c*
Leucine (α-isopropyl-α-amino propionic acid)	7.92	Levene and Van Slyke[64]	*d*
Isoleucine (β-methyl-β-ethyl-α-amino propionic acid)	1.43	Levene and Van Slyke[64]	
Proline (α-pyrrolidine carboxylic acid)	8.40	Dakin[22]	
Phenylalanine (β-phenyl-α-amino propionic acid)	3.88	Foreman[38]	*e*
Tyrosine (β-hydroxyphenyl-α-amino propionic acid)	5.70	Bissegger[9]	*f*
Glutamic acid (α-amino glutaric acid)	21.77	Foreman[38]	*g*
Aspartic acid (α-amino succinic acid)	4.10	Dakin[22]	*h*
Hydroxy glutamic acid (β-hydroxy-α-amino glutaric acid)	10.50	Dakin[22]	
Serine (β-hydroxy-α-amino propionic acid)	0.43	Fischer[31]	
Hydroxy proline (γ-hydroxy pyrrolidine α carboxylic acid)	0.23	Fischer[33]	*i*
Tryptophane (β-indol α-amino propionic acid)	1.70	Dakin[22]	*j*
Cystine (di-α-amino propionic acid ββ'-di sulfide)	0.02	Morner[73]	*k*
Arginine (α amino δ-guanidine valerianic acid)	4.84	Hart[illegible]	*l*
Citrulline (α-amino-δ-carbamido valerianic acid)		Wada[123]	*m*
Histidine (α-amino-β-imidazole propionic acid)	3.39	Bissegger[9]	*n*
Lysine (α-ε-di-amino caproic acid)	7.72	Van Slyke[120]	*o*
β-benzyl-oxy-propyl-di-hydroindolyl alanine ($C_{11}H_{14}O_3N_2$) (existence doubtful)		Abderhalden and Sickel[illegible]	*p*
Diaminotrihydroxydodecanic acid ($C_{12}H_{26}N_2O_5$ or $C_{12}H_{24}N_2O_4 \cdot H_2O$) (existence doubtful)	0.75	Fischer and Abderhalden[34]	
Methionine (γ-methylthiol-α-amino-*n*-butyric acid)	1.40	Pirie,[81] Barger and Coyne[132]	
?-Amino butyric acid ($C_4H_9NO_2$)		Foreman[37]	
β-hydroxy-α-amino butyric acid		Rimington[87]	
A diamino acid ($C_2H_6N_2O_2$) (existence doubtful)		Drechsel[26]	
Ammonia	1.61	Osborne and Harris[76]	
Phosphorus	0.85	Hammarsten[50]	*q*
Sulfur	0.76	Hammarsten[50]	
Carbohydrate (galactose) (possibly present)		Sørensen,[103] Tillmans[107]	
Sum of all products isolated so far	97.83		
Theoretical yield of decomposition products	110 to 120		

Notes to Table 2:

a Also reported as 1.50 per cent.[149]

b Also reported as 7.20 per cent.[149]

c This value is probably too low. Abderhalden and Reich suggest that at least 1 per cent is present.

d Also reported as 10.5 per cent.[130]

e Also reported as 3.2[130] and 5 per cent.[139]
f Also reported as 4.5,[130] 7.49,[138] and 4.5 per cent.[136]
g Also reported as 15.55 per cent.[149]
h Also reported as 1.39 per cent.[149]
i Fürth and Minnibeck,[43] by an isolation method combined with amino and non-amino nitrogen, report 5.7 per cent proline and 2.1 per cent hydroxy proline.
j Also reported as 1.5 [130] and 2.23 per cent.[138]
k This value is undoubtedly too low. Folin and Looney[36] by a colorimetric method report 0.25 per cent and Okuda[75] by another colorimetric method reports 0.17 per cent. Teruuchi and Okabe[153] by a modification of Okuda's method report 0.33 per cent. Sullivan[104] using his specific colorimetric method reports 0.28 to 0.30 per cent. Vickery and White[154] report 0.21 per cent and Jones and Gersdorff[138] report 0.34 per cent.
l Osborne, Leavenworth and Brautlecht[77] report 3.39 per cent arginine and 2.50 per cent histidine, values nearer those found by other workers than are the results of Hart[52] or Bissegger,[9] although Furth and Deutschberger[42] by a somewhat indirect method report 5.2 per cent arginine. Vickery and White[155] report 3.85 per cent arginine and Jones and Gersdorff[138] 5.81 per cent.
m The amount present is not stated.
n Also reported as 2.50,[77] 3.64,[138] 1.83,[155] and 2.61 per cent.[136]
o Also reported as 5.18,[138] 6.25,[155] and 5.77 per cent.[142]
p Originally identified as hydroxy tryptophane by Abderhalden and Kempe.[2]
q Bosworth and Van Slyke[133] reduced the phosphorus content to 0.70 per cent by treating an ammoniacal solution of casein with ammonium oxalate to precipitate traces of calcium phosphate.

Table 2 shows the maximum yields of crystalline amino acids that various investigators have isolated from casein by boiling with mineral acids. Altogether they total nearly 98 per cent, showing that the content of amino acids is probably better known in casein than in any other protein of common occurrence or of similar complexity, with the possible exception of zein, where the summation slightly exceeds 101 per cent. There is still an appreciable fraction of the casein unaccounted for, however, because the theoretical recovery should lie somewhere between 110 and 120 per cent of the dry casein; the amino acids result from hydrolysis of the protein, water entering into chemical reaction at the peptide linkages, R—NH—CO—R′ + H_2O = R—NH_2 + HOOC—R′, so that the amino acids isolated from a protein materially outweigh the original protein.

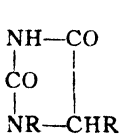
The very unsatisfactory state of our methods for quantitative determination of the amino acid content of proteins is strikingly illustrated by the relatively recent isolation by Dakin[22] of a new amino acid, β-hydroxy glutamic acid, in casein. The surprising part of the discovery was the fact that the new amino acid amounted to 10.5 per cent of the weight of the casein. Further evidence of our imperfect knowledge of the organic chemistry of casein and of proteins in general is afforded by Mueller's[74] late discovery of a new sulfur-containing amino acid, methionine, in casein, Abderhalden and Reich's[3] isolation of norvaline, and Wada's[123] isolation of citrulline from casein.

The diaminotrihydroxydodecanic acid of Fischer and Abderhalden[34] and the hydroxy-tryptophane of Abderhalden and Kempe[2] have been reported from no protein except casein and their actual existence in casein is extremely doubtful, although Fränkel and Friedmann[39] recently isolated what appears to be Fischer's $C_{12}H_{26}N_2O_5$ acid. They conclude that one molecule of water of crystallization is present, that all the nitrogen is in the form of free amino groups, and that two carboxyl groups are indicated. The compound therefore would have the formula $C_{10}H_{18}(NH_2)_2(COOH)_2.H_2O$. A determination of molecular weight agreed well with the calculated value.

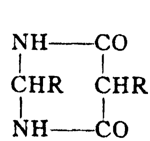
Abderhalden and Sickel[5] on reisolation concluded that the compound designated "hydroxy-tryptophane" was not such, but may be β-benzyl-oxypropyl-di-hydroindolylalanine ($C_{11}H_{14}O_3N_2$); still later[6] they concluded that it is a peptide composed chiefly of tyrosine and proline. Drechsel[26] reported a diamino acid of the formula $C_2H_6N_2O_2$ isolated from casein but not as yet from any other protein: the isolation has not been confirmed by other workers. Drechsel believed this to be a diamino acetic acid, but Willstätter[127] points out that its properties are not those of the diamino acetic acid that he synthesized. The existence of this amino acid is problematical.

Aside from the amino acids actually isolated from casein there is evidence of other compounds not as yet isolated. Thus Skraup and Krause[101] reported 0.85 per cent of methyl groups in the form of methoxy ($—OCH_3$) derivatives and 1.13 per cent of methyl groups attached to nitrogen. Geake and Nierenstein[45] reported 0.88 per cent of total CH_3 groups, of which 0.33 to 0.38 per cent represents $N—CH_3$ linkages. Herzig and Landsteiner[54] found 0.64 per cent methoxy-methyl and 1.78 per cent N—methyl groups and Burn[15] found 0.77 per cent of $N—CH_3$ methyl groups. No methoxy or N—methyl compounds have ever been isolated from a protein. The $—S—CH_3$ group in methionine may be in a considerable measure, if not wholly, responsible for the results reported by these earlier workers.

Dunn[28] pointed out that carbon dioxide is liberated during the acid hydrolysis of casein. The carbon dioxide liberated in a 24-hour hydrolysis corresponded to 0.71 per cent of the weight of the casein; 0.31 per cent was liberated in the first 5 hours. He suggested that the source of the carbon dioxide may be uramino acids, uramino acid anhydrides (hydantoins) or cyclic diacipiperazines, possibly in such structures as



or



Acetaldehyde as a Casein Decomposition Product

Hansen and Nagel[51] obtained acetaldehyde in yields of 1.51 to 1.74 per cent from the alkaline hydrolysis of casein. The yield was constant when the NaOH concentration varied between 0.5 and 10.0 per cent and the yield was somewhat greater when the distillation was performed in an atmosphere of nitrogen rather than of air. They accordingly concluded that the acetaldehyde is not an oxidation product, nor does it arise from carbohydrates. When the protein had previously been hydrolyzed with acids and the hydrolytic products subjected to the alkaline hydrolysis, no acetaldehyde was obtained. Possibly this is due to the fact that the aldehyde, if freed by acid hydrolysis, would promptly unite with the tryptophane (or tyrosine),[46] and thus disappear. Hansen and Nagel concluded that the acetaldehyde probably is formed from some as yet unknown type of linkage in the protein molecule.

Question of Pyridine or Piperidine Ring Compounds in Casein

Troensegaard has long insisted that there are pyridine derivatives, or the pyridine ring, in proteins. One argument is the common occurrence of pyridine in fermentation amyl alcohol. Troensegaard and Mygind[110] acetylated casein and then reduced the acetylated product. The hydrogenated product was then hydrolyzed and piperidine isolated. They concluded that the piperidine probably arose from a piperidine ring in the protein, not from a pyridine nucleus. Two other bases having the formulas $C_{11}H_{22}N_2$ and $C_{14}H_{22}N_2$, the nature of which has not as yet been determined, were also isolated.

Question of a Carbohydrate Group in Casein

Tillmans and Philippi[107] and Sørensen and Haugaard[103] on the basis of colorimetric and photometric methods, concluded that casein contains a carbohydrate residue. Sørensen and Haugaard identified it as galactose and reported 0.31 per cent in their sample of commercial "casein *nach* Hammarsten." It is questionable if this is an integral part of the casein molecule; more likely it is adsorbed by the casein during preparation. Tillmans and Philippi reported that the content of carbohydrate dropped from 0.89 per cent in casein reprecipitated three times to 0.36 per cent when the casein was dissolved and precipitated a fourth time. This 0.36 per cent agrees well with the 0.31 per cent reported by Sørensen and Haugaard, but does not prove that still more solution and reprecipitation of the casein would not further reduce the carbohydrate content. The subject requires further investigation before final conclusions can be drawn.

Nitrogen Distribution in Casein

Table 3 shows the distribution of nitrogen in casein from cow's milk as determined by several investigators using Van Slyke's method.[119]

TABLE 3.—Nitrogen Distribution in Casein as Determined by the Van Slyke Analysis, Expressed in Per Cent of Total Nitrogen

	Van Slyke[118]	Van Slyke[120]	Crowther and Raistrick[20]	Dunn and Lewis[29]	Hoffman and Gortner[56]	Jones and Gersdorff[138]
Ammonia nitrogen	10 43	10 27	10 25	10 49	10 20	11 53
Humin nitrogen	3 43	1 28	1 20	2 13	1 51	1 27
Cystine nitrogen	1 95	0 20	1 24	0 48	1 05	0 89
Arginine nitrogen	7 51	7 41	9 22	7 42	9 20	11 60
Histidine nitrogen.	4 24	6 21	6 82	6 01	6 26	6 12
Lysine nitrogen. .	7 86	10 30	9 62	9 09	8 49	6 17
Amino nitrogen in filtrate from bases	55 04	55 81	54 76	58 78	54 12	59.77
Non-amino nitrogen in filtrate from bases	9 51	7 13	7 09	5 93	8 76	2 63
Total. .	99 97	98 61	100 19	100 33	99 59	99 98

The ammonia nitrogen is usually ascribed chiefly to acid amide linkages, R—CO—NH_2, present in the protein and believed to be largely associated with the dicarboxylic acids. A small part of the ammonia nitrogen probably originates in deaminization under the influence of the acid used to hydrolyze the protein.[47] The humin nitrogen is in a large measure derived from tryptophane.[46] Van Slyke's nitrogen distribution method gives definite information about four amino acids only, arginine, histidine, lysine, and cystine. That the values for arginine, histidine and lysine reported in Table 2 are essentially correct is shown by their close agreement, in terms of percentage of total nitrogen, with the figures for these amino acids given in Table 3. Thus 4.84 per cent of arginine is equivalent to 9.86 per cent of the total nitrogen, 3.39 per cent of histidine to 5.81 per cent of the total nitrogen, and 7.71 per cent of lysine to 9.35 per cent of the total nitrogen. The cystine as determined by the Van Slyke method under the conditions of hydrolysis employed by Hoffman and Gortner[56] should represent about 65 per cent of the true cystine nitrogen.[48] If this is true the cystine (as amino acid) content of casein approximates 2.19 per cent, a value that is undoubtedly too high (see note *k* to Table 2). Probably some compound other than cystine is precipitated by phosphotungstic acid. Possibly methionine accounts for more of the sulfur in casein than does cystine. The high values of "cystine nitrogen" by the Van Slyke method still await explanation.

Phosphorus in Casein

For many years the form in which the phosphorus is present in casein was uncertain although it was believed to be phosphoric acid. Among proteins casein is a relatively strong acid; its titration curve with sodium

hydroxide is sharply inflected between pH 6.0 and 7.5, where the secondary hydrogen of phosphoric acid is likewise neutralized.[57] Figure 1 shows such a titration curve for casein as contrasted with similar curves for gliadin, the alcohol-soluble protein of wheat, and of orthophosphoric acid.

Apparently Posternak[83] first suggested that the phosphoric acid in casein is esterified on an —OH grouping. He fractionated tryptic

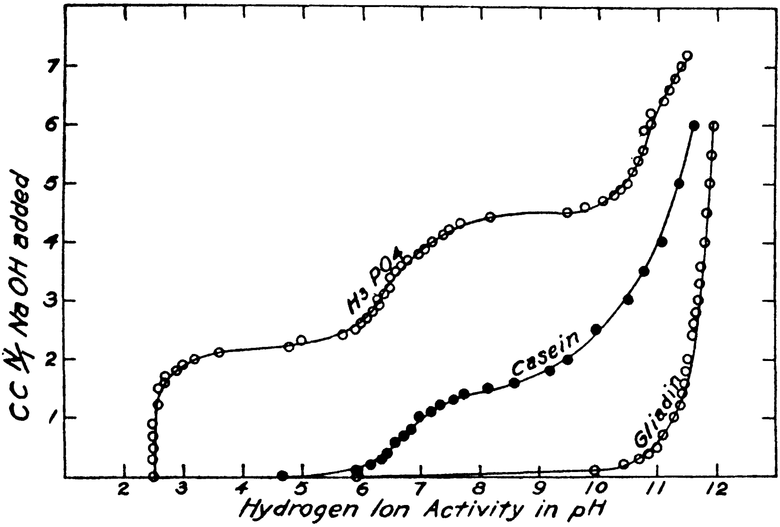


Figure 1.—The electrometric titration curve of casein as contrasted with similar curves for orthophosphoric acid and gliadin, the alcohol-soluble protein of wheat. (The data from which these curves were drawn are given by Hoffman and Gortner.[56, 57])

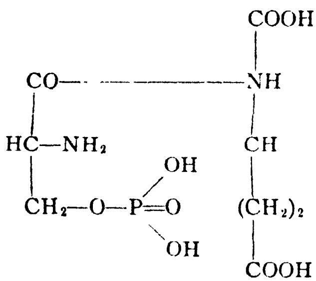
digests of casein and isolated three products which he designated α-, β-, and γ-lactotyrines having respectively the formulas $C_{64}H_{111}N_{15}O_{43}P_4$, $C_{67}H_{116}N_{16}O_{44}P_4$, and $C_{72}H_{124}N_{18}O_{47}P_4$. These compounds were soluble in water, yielding solutions strongly acid to litmus, and formed stable soluble salts with the alkalies or alkaline earths. Analyses showed the α-lactotyrine to be a complex polypeptide hydrolyzing into glutamic and aspartic acids, isoleucine and serine. He suggested the following reaction to account for the products of hydrolysis:

$$C_{64}H_{111}N_{16}O_{43}P_4 + 18\,H_2O \longrightarrow$$
$$3\,C_5H_9NO_4 + C_4H_7NO_4 + 4\,C_6H_{13}NO_2 + 7\,C_3H_7NO_3 + 4\,H_3PO_4$$

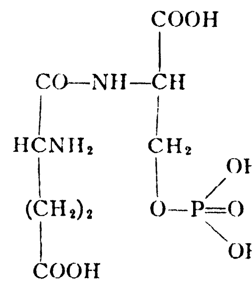
and further suggested that the phosphoric acid was linked to the serine molecules. This conclusion was later confirmed by Posternak and Posternak.[84]

Almost simultaneously Rimington[87] isolated from a tryptic digest of casein a phosphopeptone that had the formula $C_{37}H_{62}O_{33}N_9P_3$ in a yield equal to 50 per cent of the organic phosphorus of casein. There was only one free amino group in this peptone. The peptone titrated as a nine-basic acid. Analyses indicated that the only amino acids present were hydroxy glutamic, hydroxy-amino butyric and serine. Rimington presented a formula consisting of three molecules of hydroxy glutamic acid, four of hydroxy-amino butyric acid, two of serine and three of phosphoric acid. Two molecules of the phosphoric acid were attributed to mono-esters of hydroxyl groups and one molecule to a di-ester involving two hydroxyl groups of different hydroxy-amino acid molecules. Rimington thought that this formula accounted completely for the composition and properties of his phosphopeptone. Obviously his phosphopeptone is not identical with the products prepared by Posternak.

The mode of linkage of at least a part of the phosphoric acid was definitely solved when Levene and Hill[63] and Schmidt[95] almost simultaneously but independently reported the isolation of a phospho-dipeptide, glutamyl-serine-phosphate, which according to Levene possesses one or the other of the following alternative formulas:



or



The fact that the phosphoric acid was esterified on the alcoholic group of serine was further proved by the isolation by Lipmann[71] of serine phosphoric acid from casein following hydrolysis by boiling with 2.5*N* hydrochloric acid.

It is difficult to reconcile Rimington's findings with those of Posternak, Levene and Hill, and Schmidt. Rimington reported complete absence of glutamic acid in his phosphopeptide; in fact he was able to isolate only β-hydroxy-amino acids. He did demonstrate that the phosphorus in casein probably is bound in two different ways. Thus he found that bone phosphatase, which has very little action on the di-esters of phosphoric acid, removed only two-thirds of the phosphorus from his phosphopeptone, whereas kidney phosphatase liberated the remaining third. He found a similar 2: 3 ratio for the action of bone and kidney phosphatase on the whole tryptic digest of casein and concluded that his compound is representative of the entire molecule. Possibly the

experimental conditions under which Rimington worked differed sufficiently from those of Levene and Hill and of Schmidt to cause hydrolysis at two different peptide linkages, one of which gave a product free from glutamic acid and the other a dipeptide phosphate containing glutamic acid.

Paranuclein and Paranucleic Acids from Casein

Hammarsten[50] digested casein *in vitro* with pepsin and found that a precipitate rich in phosphorus separated. This precipitate he believed to be a nuclein. It was insoluble in water or in dilute (0.2 per cent) hydrochloric acid, or in dilute acetic acid. He stated that the nuclein is present in the casein as an integral part of the casein molecule and is split off during peptic digestion.

Malfatti[72] later prepared the same product by peptic digestion of casein and found that it dissolved readily in ammoniacal solutions. He was, however, unable to isolate any purine bases after hydrolyzing with dilute sulfuric acid. To prevent confusion with the true nucleins which do contain purine bases he suggested that this compound and other compounds of a similar nature should be called *paranucleins* or *para nucleic acids*. Plimmer and Bayliss[82] studied the action of pepsin, trypsin, and papain on casein, with special reference to the fate of the phosphorus. They found that trypsin converted practically all the phosphorus into a soluble form (*i.e.*, not precipitable by tannin) within 24 hours, and that the "soluble P_2O_5" consisted of approximately 35 per cent of phosphoric acid and 65 per cent of organic phosphorus compounds. Pepsin, on the other hand, even after digestion for 146 days, converted only about 70 per cent of the phosphorus into a "soluble form"; and even then the soluble phosphorus was almost wholly in organic combination. The action of papain was intermediate between that of trypsin and pepsin.

Salkowski,[91] Salkowski and Hahn,[93] Alexander,[7] Reh,[86] Dietrich[25] and others investigated the compounds containing phosphorus which can be isolated from the peptic digests of casein.

Salkowski's preparation, which he called *para nucleic acid,* was soluble in water, forming a solution sufficiently acid to decompose carbonates of the alkalies or alkaline earths, was insoluble in alcohol and acetic acid, levorotatory ($\alpha_D = -46°$), in aqueous solution precipitated aqueous solutions of egg albumin, lead acetate, copper acetate, tannin, and phosphotungstic acid in hydrochloric acid. Analysis gave: C = 42.51 per cent, H = 6.97 per cent, N = 13.25 per cent, and P = 4.31 per cent.

Reh's product was prepared by precipitation with uranyl acetate and contained eight times the phosphorus content of casein (6.9 per cent: 0.83 per cent P). After hydrolysis of 380 grams of the uranyl

precipitate containing approximately 200 grams of organic material, Reh separated glutamic acid (13.4 grams), aspartic acid (1.0 gram), lysine (7.0 grams), isoleucine (1.5 grams), amino valerianic acid (1.4 grams), leucine (1.3 grams), proline (0.9 gram), histidine (0.4 gram), phenylalanine (0.1 gram), and alanine (0.1 gram). Tyrosine was present as indicated by Millon's reaction. Reh suggested that his preparation was a mixture of two or more acids having the structure of a large polypeptide united to one of the hydrogens of phosphoric acid.

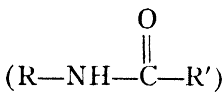
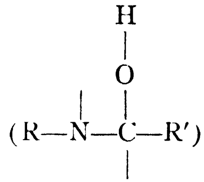
Dietrich confirmed this suggestion by fractionating the uranium precipitate with zinc, copper, and lead salts. At least four different products resulted, containing respectively 10.0, 4.1, 3.84, and 3.88 per cent phosphorus. The corresponding N/P ratios were 1 : 1, 3.07 : 1, 2.59 : 1, and 3.87 : 1. The product having the N/P ratio 1 : 1 was not a simple amino acid phosphate, as the ratio might suggest, but contained at least proline, glutamic acid, and lysine. The quantities of these three amino acids failed to account for all of the molecule so that other amino acids were probably present.

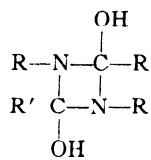
Holter, Linderstrøm-Lang and Funder[58] prepared a series of casein fractions in which the ratio P/N ranged from 0.0022 to 0.0523, and digested them with pepsin under rigidly controlled conditions, at pH 2.30. The phosphopeptone separated as a white voluminous gel which was removed in a centrifuge and the ratio P/N in the precipitate determined. The ratio P/N in the phosphopeptone was the same (range for different experiments 0.105 to 0.122) regardless of the ratio P/N in the initial casein. This substance "is not identical with the peptone obtained by tryptic digestion described by other workers."

It is difficult to believe that anyone, with the possible exception of Holter, Linderstrøm-Lang and Funder, has actually isolated from a casein-pepsin digest a true "nuclein" or "para nuclein" in the sense of a specific nucleus of the original casein containing phosphoric acid. In general the products noted above are probably mixtures of various peptones and peptides containing phosphorus; the divergent opinions and analyses are due to different experimental conditions, including different proteolytic activity of the pepsins.

Complex Polypeptides and the Intra-Molecular Structure of Casein

Fodor and Kuk[35] present data on products made by heating casein for three hours at 130 to 150°C. in anhydrous glycerol or resorcinol. Their conclusions do not follow convincingly from their data. They reported the isolation of a series of "acropeptides" having the formulas $C_{41}H_{67}N_9O_{12}$, $(C_{41}H_{67}N_9O_{12})_2$ and ½ $(C_{41}H_{67}N_9O_{12})$, the first being a di-tetra-peptide, the second a tetra-tetra-peptide and the last a simple tetra-peptide.

The tetra-tetra-peptide is said to consist of two molecules of proline, two molecules of pyrrolidin-carboxylic acid (formed from glutamic acid by ring closure), two molecules of leucine and one molecule each of alanine and lysine. Structural formulas for such compounds are proposed which involve a novel linkage between the tetra-peptide groups. This linkage results from a tautomeric shift in the ordinary peptide linkage  to  releasing two free valences that permit two molecules to unite in the ring



Reversal of the process causes the molecule to "dissociate" into smaller complexes. They suggest that such "combination" and "dissociation" is common in proteins and that approximately 50 per cent of casein and 35 per cent of gelatin is composed of the "acropeptides." The theory is reminiscent of Robertson's[90] theory of dissociation at the peptide linkage.

Cherbuliez and De Mandrot[17] note that proteins in general are soluble in molten acid amides. When a suspension of casein in molten acetamide was heated, a clear solution was formed at 160 to 170°C. At 180 to 190°C. some ammonia was evolved and the melt became somewhat acid. After heating to 200°C. the melt was cooled and the acetamide extracted. The protein that remained had essentially the same elementary analysis, color reactions, and amino acid distribution as the original casein, but its molecular weight was low, 247 to 396. Preparations fractionated with alcohol varied in molecular weight from 250 to 620. Some carboxyl groups were set free by the heat treatment but the amino groups were not. The authors believed that the open-chain molecules in casein were broken and the fragments converted into closed-chain molecules of lower molecular weight. This may be a new technic for studying protein structure.

Question of the Identity of Caseins from Milk of Different Mammals

As shown in Table 1, the elementary analyses of the caseins isolated from the milk of different mammals are so nearly identical that they do

not serve to differentiate the sources of the caseins. The physical properties, insofar as they have been compared, likewise point to close relations if not actual identity.

Abderhalden and Schittenhelm[4] found a striking similarity in the content of amino acids in the caseins from cow's milk and goat's milk and in the content of tyrosine in woman's, cow's, and goat's milk. Variations in the content of amino acids in caseins from different mammals evidently do not differentiate the caseins.

The biological reactions of the proteins as practiced in immunology are generally accepted as the most delicate and accurate means of identifying a specific protein or proteins. Such tests fail to reveal differences between caseins from different mammals. Wells[124] sums up as follows: "An immune serum for any one casein will give reactions with casein from any other species, even of remote relationship," and "casein from the milk of an animal of any given species shows a closer biologic relationship to the casein of another species than it does to either the whey proteins or to the serum proteins of its own species."

Dudley and Woodman[27] from a comparative study of the optical activity of the amino acids isolated from racemized casein (*vide infra*) prepared from cow's milk and sheep's milk, found evidence of differences in the actual arrangement or order of the amino acids in the protein molecule even though the content of amino acids in the two proteins was the same. Each species may synthesize a specific casein but the differences between them may be merely the intra-molecular arrangement of the various amino acids.

Paracasein

Casein is converted into paracasein by the action of the enzyme, rennin. The literature dealing with this phenomenon is very voluminous and discordant.

Van Slyke and Bosworth[121] made comparative elementary analyses of casein and paracasein with the following results on the ash-free basis:

	Carbon %	Hydrogen %	Nitrogen %	Phosphorus %	Sulfur %
Casein	53.50	7.13	15.80	0.71	0.72
Paracasein.	53.50	7.26	15.80	0.71–0.83	0.72–0.87

Geake[44] reviewed the older literature and added analyses of his own, including elementary analyses and the distribution of nitrogen by the Hausmann method, concluding that casein and paracasein are identical in chemical composition within the limits of present methods of analysis.

Wright[128] studied the change in optical rotation in the presence of

alkali. He concluded that casein and paracasein show the same racemization curves and that the transformation of casein to paracasein is a colloidal phenomenon in which the organic chemistry of the casein molecule remains unchanged. Palmer and Richardson[78] reviewed the literature and concluded that no adequate explanation has as yet been suggested. Most of the theories postulate formation of paracasein by a physico-chemical process in which the chemical composition of the casein molecule remains unaltered. This view agrees with the more recent work. Deep-seated changes in the organic chemistry of the casein molecule can scarcely be imagined in the extremely short time required for the enzyme, rennin, to clot milk. Accordingly the organic chemistry of paracasein can be considered identical with the organic chemistry of casein.

Racemized Casein

When proteins are treated with dilute alkali and the mixture allowed to stand at room temperature (or preferably at 37°C.) for several days the optical rotation of the protein solution decreases progressively to a low but practically constant value. When such racemized proteins are hydrolyzed, certain amino acids are found to be completely inactive optically, but others retain their original activity. Dakin[21] suggested that the racemization is a tautomeric change of the keto-enol type involving the carbonyl group of the peptide linkage in the protein molecule and the hydrogen on the α-carbon atom of the same amino acid.

$$\text{—CO—NH—}\overset{\displaystyle \text{R}'}{\overset{|}{\text{C}}}\text{H—CO—NH—CHR—COOH} \longrightarrow \text{—CO—NH—}\underset{\displaystyle \text{R}'}{\underset{|}{\text{C}}}\text{=C(OH)—NH—CHR—COOH}$$

Inasmuch as the α-carbon atom is asymmetric, formation of the double bond destroys its optical activity and later rupture of the double bond by acid hydrolysis produces equal quantities of the *d* and *l* forms of the amino acid, that is a racemic mixture. Certain amino acids in the protein molecule may remain unracemized because "the conditions necessary for racemization of an amino group require the attachment of other groups to both amino and carboxyl radicle," so that amino acids in terminal positions in the peptide chains of the protein molecule still retain their optical activity.*

Dakin and Dudley[23] prepared racemic casein by dissolving 500

* Kober[141] thought that the double bonds between carbon atoms formed by the enolization postulated by Dakin should be revealed spectrographically by specific absorption, of which he found no evidence experimentally. Groh and Weltner,[135] however, later showed that enolization can occur without being detected spectrographically.

grams of commercial casein in 5 liters of 0.5*N* sodium hydroxide and incubating for 18 to 20 days at 37°C. The optical rotation fell from about –5° to about –3°. The racemic casein was precipitated by acidification with glacial acetic acid and the resulting product, which was plastic and dough-like, was washed repeatedly with water until the wash water was neutral to litmus. After drying for several days at room temperature a light-brown, friable mass was obtained which could be reduced to a fine white powder, closely resembling the original casein. Elementary analysis gave carbon 53.55 per cent, nitrogen 12.5 per cent, hydrogen 7.03 per cent.

The yield of racemized casein was only about 20 per cent of the original casein. This and the facts that considerable ammonia was evolved during the incubation and that racemized proteoses remained in the mother liquor following the precipitation of the racemic casein, all point to extensive protein hydrolysis accompanying the tautomeric change responsible for the reduced optical rotation.[134]

Dakin and Dudley[24] found that racemized casein was not digested by pepsin, trypsin, or erepsin when tested *in vitro*, nor was it digested when fed to dogs. Following the feeding, unchanged racemic casein was recovered from the feces. The racemic casein also resisted the attack of putrefactive bacteria during a 10-day period.

Levene and Bass[62] studied the racemization of casein with particular reference to the rate of racemization as affected by the concentration of the alkali used. They point out that dilute alkali (0.1 to 0.2*N*) acts slowly on true polypeptides whereas it rapidly produces racemization of the amino acids in ketopiperazine rings. Stronger alkali (0.5 to 1.0*N*) causes hydrolysis and slow racemization of peptides, but very rapid hydrolysis and very little racemization of amino acids liberated from ketopiperazines. Racemization of the amino acids *precedes* hydrolysis, so that, from readings of the optical rotation and the ratio of amino to total nitrogen, the rates of both racemization and hydrolysis can be followed. These investigators conclude that casein is not composed of ketopiperazines *of the type so far studied* and that it does not behave like the simple polypeptides that they studied. The rate of racemization was more nearly that expected for ketopiperazines, but the rate of hydrolysis was more nearly that for polypeptides. Even at 125°C. in 1.0*N* alkali, racemization was complete, as was hydrolysis, in two hours. Casein is either an unusually stable ketopiperazine, or else the order of linkage of its amino acids favors racemization.

The Action of Halogens on Casein

It is doubtful whether the numerous preparations described as resulting from the action of halogens upon casein represent simple sub-

stitution products; probably partial hydrolysis has taken place simultaneously. The halogen apparently enters by substitution in the aromatic nuclei of the protein molecule, inasmuch as Millon's reaction (for tyrosine) and Adamkiewicz' reaction (for tryptophane) are invariably negative when the halogenated product is tested.

Chlorine

Habermann and Ehrenfeld[49] chlorinated casein by dissolving it in 5 per cent potassium hydroxide, adding potassium chlorate and bubbling hydrogen chloride into the mixture held at room temperature until complete solution took place. The chlorinated product was precipitated by diluting with water and purified by repeated solution and reprecipitation. Upon drying it became brown and lost its solubility in water. Analysis showed 13.28 to 14.04 per cent chlorine.

Panzer[79] prepared a chlorinated product by adding 450 grams of potassium chlorate to a mixture of 1,000 grams of casein suspended in 4,000 grams of 20 per cent hydrochloric acid. No attempt was made to control the temperature and the mixture became "very warm." The mixture was allowed to cool and was then greatly diluted with water and the insoluble portion washed with distilled water until free from chlorides. A cream-colored powder weighing 770 grams resulted. It was insoluble in water or neutral organic solvents, readily soluble in dilute alkalies and reprecipitated by acids. Elementary analysis gave: carbon 47.05 per cent, hydrogen 5.52 per cent, nitrogen 12.40 per cent, sulfur 0.23 per cent, phosphorus 0.81 per cent, chlorine 8.32 per cent, oxygen 25.67 per cent.

When this product was hydrolyzed with hydrochloric acid, leucine, glutamic acid, aspartic acid, arginine, histidine, and lysine were identified among the hydrolytic products. In a later paper Panzer[80] described a series of products obtained by the action of sodium ethylate upon the chlorinated casein. Apparently he was able to replace a part of the chlorine by the ethoxy group. Salkowski[92] prepared a chlorinated casein by treating 5 grams of casein with 5 grams of sodium chlorate dissolved in 125 cc. of water and adding 20 cc. of hydrochloric acid (1.126 specific gravity). The mixture was heated over a free flame until the liquid became lemon-yellow in color. An excess of sodium hydroxide was added and, after cooling, the chlorinated product was precipitated by acidification with hydrochloric acid. The light-yellow precipitate was filtered and washed, first with water and later with alcohol followed by ether. When dry it formed a fine white powder. Tests for tryptophane and tyrosine were negative. A faint xanthoproteic reaction was obtained indicating that some unchlorinated phenylalanine still remained in the molecule. Analyses gave 6.72

to 6.78 per cent chlorine and 0.85 per cent phosphorus. The chlorinated product was appreciably soluble in hot water but was reprecipitated by the addition of sodium chloride to the solution. It was likewise quite soluble in hot 50 per cent alcohol, was very resistant to the action of pepsin or trypsin *in vitro* and was only slightly attacked by putrefactive bacteria. No indol formation was observed when the chlorocasein was acted on by indol-producing bacteria.

Vandervelde[117] recently prepared a chlorocasein by suspending dried and finely pulverized casein in carbon tetrachloride and adding chlorine gas until the weight of the final chlorinated product no longer increased. This was then washed with carbon tetrachloride and dried. It formed a light yellow powder, had an irritating odor, and was somewhat hygroscopic. It contained 10.6 per cent nitrogen and 31.9 per cent chlorine. When treated with cold water it decomposed into soluble and insoluble fractions, the amount of each and the chlorine content depending upon the duration of contact with water, temperature, time, etc.

Bromine

Hopkins and Pinkus[59] prepared a number of halogenated proteins. Apparently the only preparation made from casein was the bromine derivative which contained 11.17 per cent bromine. Its properties and reactions are not described separately. In general the products have the following properties: the xanthoproteic and biuret reactions are positive, Millon's and Adamkiewicz' reactions are negative, as is the reaction for loosely bound sulfur. The halogenated proteins are quite soluble in hot alcohol, and difficultly soluble in cold alcohol, water or neutral organic solvents. The halogen derivatives are relatively strong acids, decomposing carbonates and forming stable compounds with the heavy metals, especially lead, mercury, and silver. The products are soluble in dilute alkalies and precipitated by neutralization of these solutions with acetic acid.

Vandervelde[116] prepared a bromocasein through methods similar to those by which he prepared chlorocasein. He reports nitrogen contents of 9.6 to 10.1 per cent and bromine contents of 32.2 to 35.0 per cent. In comparison with chlorine in the chlorocasein, only approximately half as many atoms of bromine were combined in the bromocasein. The bromocasein had properties very similar to the chloro-compound. There may be insufficient aromatic radicals to account for the substitution of halogens in the casein; halogen acids were evolved in small proportions only; therefore the halogens must enter at double bonds or on residual valences on nitrogen atoms.

Bromine compounds are described also by Lieben and Müller [144] and by Yaichnikov.[158]

Iodine

Liebrecht[70] described several products of the action of iodine on casein. "Periodiocasein" was obtained by stirring an intimate mixture of 80 grams of casein and 20 grams of iodine heated to 100°C. on a water bath, and later extracting the brown powder with ether until the ether became colorless. The residual ether-insoluble product contained 17.8 per cent iodine. It is a yellow powder soluble in hot dilute alcohol and reprecipitated as brown flocks on cooling. A part of the iodine is loosely bound and can be removed by treatment with sodium thiosulfate.

"Iodiocasein" resulted from the treatment of the "periodiocasein" with sodium thiosulfate. It is a white powder, insoluble in neutral organic solvents and is appreciably acid in reaction, dissolving readily in dilute alkalies and being reprecipitated by acids. It contains both phosphorus and sulfur and according to Liebrecht "can be separated from casein by being insoluble in sodium sulfite solution." "Iodiocasein" contained 5.7 per cent iodine.

A third product, "caseoiodin," containing 8.7 per cent iodine, was prepared by heating 100 grams of "periodiocasein" for two hours with 2,000 cc. of 10-per cent sulfuric acid on a water bath. The insoluble residue was repeatedly dissolved in alkali and reprecipitated with acid followed by a final extraction with hot 70-per cent alcohol. The "caseoiodin" separated from the alcohol on cooling as colorless flocks which when dried with alcohol and ether formed a white powder.

Iodine compounds are described also by Lieben and Lazzlo,[145] Yaichnikov,[157] Lepinois,[143] and Masui.[147]

Desaminocasein

Skraup and Hoernes,[100] Levites,[66] Dunn and Lewis,[29] Herzig and Lieb,[137] Lewis and Updegraff,[67] Nakashima,[148] Steudel *et al.*,[152] Wiley and Lewis,[126] and White[156] prepared and studied desaminocasein. It is prepared by the action of nitrous acid on casein. Dunn and Lewis suspended 100 grams of casein in 2 liters of water and slowly added 140 cc. of glacial acetic acid followed by 500 cc. of an 8-per cent solution of sodium nitrite. Vigorous mechanical agitation was used during the entire process. After standing 18 hours at room temperature the precipitate was filtered on a Buchner funnel and washed repeatedly with hot water followed by a thorough washing with alcohol and later ether, and dried at 80°C. The yield varied from 90 to 97.5 grams of a fine yellow powder. The product slowly dissolved in 1.5-per cent sodium hydroxide to a red solution. The Hopkins-Cole, Millon, and biuret tests were positive. The nitrogen content was from 0.22 to 0.68 per cent lower than that of the original casein. The free amino

nitrogen (derived largely from the ϵ-NH_2 group of lysine) was greatly reduced, in one sample being entirely absent. Tyrosine is apparently partially destroyed by the action of the nitrous acid. Arginine is apparently unaffected but a part of the histidine is destroyed. The content of tryptophane is unchanged and in other respects the protein appears to be but slightly altered. The nitrogen distribution corresponds very closely to that of the original casein when allowance is made for the total absence of lysine due to the removal of its ϵ-NH_2 group.[29, 94]

The action of proteolytic enzymes on deaminized casein[30] gave a product that was attacked *in vitro* directly by erepsin only after a preliminary digestion with pepsin or trypsin. The digestion by pepsin or trypsin proceeded much less rapidly than with unaltered casein as the substrate. *In vivo* experiments showed that desaminocasein is digested and metabolized in the animal body.

Nitro-casein

The xanthoproteic reaction of proteins is conducted by treating the protein with concentrated nitric acid, thereby producing a yellow color. It has long been known that the reaction depends upon aromatic nuclei in the protein molecule. Inouye[60] nitrated silk fibroin and isolated a nitro-tyrosine from the hydrolytic products of the nitro protein. Johnson[61] later showed that the nitro group was in position 3, *i.e.*, 3-nitro-4-hydroxy-phenyl-α-amino propionic acid. According to both Inouye and Johnson, this compound is responsible for the xanthoproteic reaction.

Von Furth[41] nitrated casein at room temperature in the presence of urea. The urea was added to eliminate the effect of nitrous acid on the course of the reaction. The *nitro-casein* thus obtained was a bright yellow powder soluble in alkalies to a red brown solution, and precipitated by acetic acid. Millon's reaction and the lead sulfide reactions were not given by this product.

In the absence of urea during nitration von Furth obtained a second product, *xanthoprotein*. This was apparently very similar to nitro-casein, but was probably the product of more drastic hydrolysis or decomposition. The ratio of $S:NO_2:N$ in the xanthoprotein was 1:1.5:44. Trypsin slowly digested the nitro-casein, forming nitrated proteoses and peptones.

Methylated Casein

Skraup and Krause[101] were apparently the first to methylate casein. Fifty grams of casein was suspended in 100 cc. of absolute alcohol and a solution of 13 grams of potassium hydroxide dissolved in 25

cc. of absolute alcohol was slowly added with constant agitation. The casein dissolved in the alkaline alcohol so that a solution was obtained which was only slightly turbid. To this solution 50 grams of methyl iodide was added and the mixture heated under a reflux condenser for approximately 2 hours or until the alkaline reaction disappeared. An additional 50 grams of methyl iodide and 13 grams of potassium hydroxide were then added and the mixture again heated until the alkaline reaction disappeared. The alcohol, together with any unchanged methyl iodide, was distilled off, the residue extracted with 500 cc. of boiling water and the soluble methylated casein purified by repeated precipitation with ammonium sulfate. Following the sixth precipitation with ammonium sulfate exactly enough barium hydroxide was added to remove the sulfate ions as barium sulfate and the barium- and sulfate-free solution was then evaporated to dryness *in vacuo.*

The product so prepared was a faintly yellow powder, reacting slightly acid toward litmus; the biuret and glyoxylic acid reactions were strongly positive but Millon's reaction was negative. Analyses showed iodine in the final product. Accordingly it cannot be looked upon as a simple methylated protein. The substance analyzed as follows: carbon 52.50 per cent, hydrogen 7.37 per cent, nitrogen 14.22 per cent, sulfur 0.61 per cent, phosphorus 0.87 per cent, iodine 5.35 per cent. Determination of ($O—CH_3$) on several different preparations gave values varying from 1.52 to 2.07 per cent and ($N—CH_3$) varied from 2.79 to 3.93 per cent.

Geake and Nierenstein[45] methylated casein using diazomethane as a reagent. Five hundred grams of dry casein was suspended in ether and treated with successive portions of 5 grams of diazomethane in ethereal solution until 75 grams of diazomethane had been added. After each addition of diazomethane the reaction mixture was allowed to stand in the dark until it became colorless. The methylation required about four months. The methylcasein was filtered from the reaction mixture, thoroughly washed with ether and dried. It formed a light white powder, indistinguishable in appearance from casein. It was soluble in dilute alkali (0.02*N*) only after long standing (20 hours) and was even more stable toward dilute acids, being peptized by dilute sulfuric acid only after several days' heating on a water bath. The methyl groups were split off by the alkaline treatment. Analyses gave carbon 55.31 per cent, hydrogen 7.32 per cent, nitrogen 15.49 per cent (Dumas), sulfur 0.800 per cent, and phosphorus 0.755 per cent. Analyses for methoxy and N-methyl groups gave 2.08 and 2.28 per cent respectively. Determinations of methoxy and N-methyl values for the original untreated casein gave 0.49 to 0.55 per cent methoxy and 0.87 to 0.88 per cent N-methyl; thus methylation increased the methyl groups in casein by 3.48 per cent.

The methyl casein gave all the usual color reactions characteristic of casein, including Millon's reaction, which would indicate that the *p*-hydroxy group of tyrosine had not been methylated.

Herzig and Landsteiner[54] also used diazomethane to methylate casein. They do not describe their methylated casein. Determinations of methoxy and N-methyl on their untreated casein were 0.64 and 1.78 per cent respectively, whereas their methylated casein yielded values ranging from 4.86 to 5.36 for methoxy and 3.85 to 5.34 for N-methyl groups.

Edlbacher[31] later methylated casein by treating a mixture of 1.5 grams of casein and 3 grams of sodium hydroxide in 100 cc. of water with 8 grams of dimethyl sulfate. He does not describe the properties of his methylated casein, inasmuch as he was interested only in the "N-methyl number" which he defines as "that value which indicates the number of methyl groups per hundred nitrogen atoms that are bound to nitrogen by exhaustive treatment with dimethyl sulfate in alkaline solution." He finds an N-methyl number of 19.7 for methylated casein.

Benzoyl Casein

Kimura[140] made benzoyl casein by treating casein with benzoyl chloride and sodium hydroxide. The nitrogen distribution in the product was similar to that in the original casein.

Vaughan's "Protein Poison" from Casein

The Vaughans[122] showed that when proteins are heated with a 2 per cent solution of sodium hydroxide in absolute alcohol, the protein molecule is hydrolyzed and can be separated into at least two fractions, one of which is insoluble in absolute alcohol and is physiologically inert, and the other soluble in absolute alcohol and possessing extremely poisonous properties when injected intravenously, intra-abdominally, or subcutaneously into experimental animals, the symptoms of death being very similar to those of anaphylactic intoxication.

Leach (quoted in references [113, 114, 122]) also prepared this "protein poison" from casein. The casein, together with 15 to 25 times its weight of absolute alcohol containing 2 per cent sodium hydroxide, was placed in a flask fitted with a reflux condenser, and the mixture was heated on a boiling water bath for one hour. The liquid was then decanted from the insoluble residue, and the residue extracted twice more in a similar manner. The liquids were then united, filtered and carefully neutralized with hydrochloric acid. After filtering from the precipitated sodium chloride, the alcoholic solution of the toxin was evaporated to dryness *in vacuo* at 40°C. The resulting product

was a dark brown scale which powdered to a light brown, highly hygroscopic powder. No analytical data on composition are given. This powder was readily soluble in water to an acid, slightly opalescent solution. The toxin diffused very slowly through collodion when dialyzed against distilled water. The biuret, Millon's, Adamkiewicz', and xanthoproteic reactions were strongly positive. Precipitates were produced by such protein precipitants as phosphotungstic acid, phosphomolybdic acid, uranyl acetate, picric acid, mercuric chloride, platinic chloride, lead acetate, etc.

Underhill and Hendrix,[113] Ringer and Underhill,[88] Underhill, Greenberg and Alu,[112] Underhill and Hjort,[114] and Underhill and Ringer[115] studied the physiological action of Vaughan's crude soluble poison. The poison possesses a greater toxicity than is shown by the ordinary proteoses and peptones. "The fatal outcome following the injection of the poison occurs in two ways, (1) a primary, due to respiratory failure within two hours following its administration, and (2) a secondary, occurring in 2 to 36 hours as the result of a progressive asthenia."[114, p 158] The intravenous injection produced a prolonged fall in arterial blood pressure, a delay in blood clotting time (normal clotting time of seven minutes, failure to clot in 24 hours when the poison had been injected) and a marked increase in blood concentration.

A careful study of the soluble protein from egg albumin[122] shows conclusively that the crude protein poison prepared as noted above must be considered a mixture from which a number of non-poisonous fractions can be separated. The toxin was finally concentrated so that 0.5 milligram injected intravenously sufficed to kill a guinea pig of 200 to 300 grams weight. The exact chemical nature of the poison is still unknown.

Formaldehyde Casein

Formaldehyde reacts with proteins to form more or less stable compounds in which the original properties of the protein are profoundly altered. Such proteins were prepared by Trillat,[109] Blum,[10] Benedicenti,[8] Schwarz,[97] and Treves and Salomone.[108] Blum suggested that the reaction is a condensation of the free amino groups with the aldehyde with elimination of water:

$$\begin{matrix} -NH_2 \\ \\ -NH_2 \end{matrix} + CH_2O = \begin{matrix} -NH \diagdown \\ CH_2 \\ -NH \diagup \end{matrix} + H_2O$$

or

$$-NH_2 + CH_2O = -N{=}CH_2 + H_2O$$

Benedicenti suggested in addition to these linkages an aldehyde-ammonia type of reaction:

$$R{-}NH_2 + HCHO = R{-}NH{-}\overset{OH}{\underset{H}{C}}{-}H$$

Incidentally he remarks that speculation about the linkages involved is idle until we know a great deal more about the actual chemical configuration of the protein molecule.

Benedicenti[8] prepared formaldehyde casein by suspending casein in five times its weight of water and adding 4 cc. of 2 per cent formaldehyde solution for each 5 grams of casein. The mixture was then allowed to stand at room temperature. At the end of 16 days, analyses showed that each gram of casein combined with 0.0057 gram of formaldehyde. The resulting product was insoluble in water, did not swell (become hydrated) when treated with dilute hydrochloric acid, nor was it soluble in the dilute acid, and was not attacked by pepsin, trypsin, rennet, or bacteria. When the formaldehyde casein was suspended in water and the mixture subjected to steam distillation until 200 grams of distillate had been collected from 5 grams of aldehyde casein, practically all of the "bound" formaldehyde was found in the distillate. The residue in the flask showed in certain tests the properties of unaltered casein, *i.e.*, it readily swelled (hydrated) in dilute hydrochloric acid and was readily digested by proteolytic enzymes. The only point noted in which it differed from normal casein was a red biuret reaction instead of the normal violet. This apparently indicates partial hydrolysis of the peptide linkages, probably caused by steam distillation. The fact that formaldehyde can apparently be removed readily from formaldehyde-protein complexes is at least suggestive that the aldehyde-ammonia reaction involving the acid amide groups may be the principal linkage of formaldehyde and proteins.

There is a reaction between chloral hydrate and casein. It is not similar to the reaction with formaldehyde but seems rather to be an adsorption.[146]

"Oxyprotosulfonic Acid"

Bondyznski and Zoja[11] oxidized casein with potassium permanganate (15 grams of permanganate in 1 liter of water for each 25 grams of protein) by allowing the mixture to stand at room temperature for 10 days. Inasmuch as the manganous oxide had not settled enough to permit a clear filtrate, alkali was added. The manganous oxide separated at once, leaving a clear supernatant liquid which was filtered off and acidified with hydrochloric acid, precipitating the "oxyprotosul-

fonic acid." This was filtered off, dissolved in dilute alkali and reprecipitated with acid. After washing to the absence of chlorides, it was dried, first at room temperature and later at 110°C. The following elementary analysis is typical of the product: carbon 52.07 per cent, hydrogen 6.81 per cent, nitrogen 14.91 per cent, sulfur 0.76 per cent, phosphorus 0.70 per cent.

Buraczewski and Krauze[14] fractionated this product with glacial acetic acid as a solvent, showing that it was not a simple compound but a mixture. Further investigations are reported by Schuberthowna,[96] who separated the original product into seven distinct fractions on the basis of differential solubilities in organic solvents.

Lieben and Bauminger[68] recently made a more detailed study of the oxidation of casein with potassium permanganate. They studied the "oxyprotosulfonic acid" fraction, the "*peroxyproteic* acid" fraction (that solution of protein oxidation and hydrolysis products resulting when the protein is oxidized with twice its weight of potassium permanganate until no protein flocks are precipitated on acidifying), as well as the effect of potassium permanganate on individual amino acids. In the "oxyprotosulfonic acid" they report complete absence of tyrosine and tryptophane, partial destruction of arginine, part of which disappears and another part of which is converted into ornithine and urea, and great stability toward oxidation of the dicarboxylic acid fraction. They find that cystine is not generally oxidized to cysteic acid and agree that oxyprotosulfonic acid is a misnomer. Data available indicate that oxidation of casein with potassium permanganate not only oxidizes but simultaneously hydrolyzes the protein molecule and that "oxyprotosulfonic acid" is simply a name given to a heterogeneous mixture obtained from a protein when certain specific laboratory processes are performed.

"Caseinkyrine"

Skraup[99] and Siegfred[98] isolated a basic substance from casein that had been partly hydrolyzed by treatment with ten times its weight of 12- to 16-per cent HCl for three weeks at 38 to 39°C. The product formed a crystalline precipitate with phosphotungstic acid but the free "kyrine sulfate" could not be obtained in crystalline form. Analysis of the sulfate indicated its formula to be $C_{23}H_{47}N_9O_8.3H_2SO_4$. The content of amino acid corresponded to one molecule of arginine, two of lysine, and one of glutamic acid. All three of these amino acids were isolated after complete hydrolysis of the kyrine.

Skraup and Witt[102] repeated Siegfred's work and were unable to agree with him that casein contained a "kyrine" nucleus. They found that the "kyrine" phosphotungstate could be fractionated either in water solution or in alcohol solutions of various concentrations into

a number of fractions showing different solubilities and chemical prpperties. When the "kyrine" fraction was converted into a picrate, they were able to isolate an appreciable amount of free lysine picrate.

Levene and van der Scheer[65] in a later repetition of Siegfred's work (unfortunately by a different experimental procedure in which they hydrolyzed at 40°C. with 12.5-per cent hydrochloric acid for five weeks) separated the kyrine fraction into two polypeptides, one of which gave a crystalline sulfate. The one forming the crystalline sulfate was apparently a tripeptide composed of lysine, oxyproline, and valine, although lysine was the only amino acid that they succeeded in isolating in any appreciable quantity.

The Action of Carbon Disulfide on Casein

Uhl[111] treated 5 grams of casein dissolved in 100 cc. of 5-per cent sodium hydroxide with 2 cc. of carbon disulfide and allowed the mixture to stand with frequent shaking for several days in a closed container. The mixture was then boiled under a reflux condenser for several hours, after which the excess carbon disulfide was allowed to distill off. A clear golden yellow solution was obtained. The reaction involved is said to be

$$R—NH_2 + CS_2 = R—NH—\overset{\overset{\displaystyle S}{\|}}{C}—SH$$

The sulfur compound was isolated by adding an ammoniacal solution of copper acetate (4 grams) and precipitating the mixture with acetone. The copper salt so formed was an amorphous brown powder containing 17.91 per cent copper, and was readily soluble in faintly alkaline solutions. No other properties of the casein preparation are described.

Pellizza[150] made a sulfurized derivative of casein by condensing casein in the cold with carbon disulfide in the presence of sodium carbonate, precipitating the product with dilute acid, and purifying by resolution in alkalies and reprecipitation with acids.

Freeman[40] showed that many of the amino acids in barium hydroxide solution readily react with carbon disulfide to form barium dithiocarbamates and that these can be isolated in high yield, *e.g.*,

$$\begin{array}{l} CH_2—COOH \\ | \\ NH_2 \end{array} + Ba(OH)_2 + CS_2 = \begin{array}{l} CH_2—CO—O \\ | \qquad\qquad\quad \searrow \\ | \qquad\qquad\qquad Ba \\ | \qquad\qquad\quad \nearrow \\ NH—CS—S \end{array} + 2H_2O$$

The free acids, however, were exceedingly unstable, decomposing as soon as freed from their salts. Apparently the decomposition on acidification was not entirely a simple reversal of the mechanism of formation of the dithiocarbamate, since appreciable quantities of hydrogen

sulfide occurred among the decomposition products. No trace of a nitroprusside reaction, which is characteristic of —SH groups, was shown by any of the barium salts or the decomposition products that were formed when the barium was precipitated as the sulfate.

In an attempt to prepare casein compounds of carbon disulfide, analogous to that reported by Uhl, casein was suspended in 0.5 per cent barium hydroxide and shaken for 12 hours with an excess of carbon disulfide. Alcohol was then added to the mixture and the precipitate thoroughly washed with water to remove the excess of alkali. It was finally washed with alcohol and dried with absolute alcohol and ether. The residual yellow-white powder contained 3.30 per cent sulfur, showing a definite increase in sulfur content over the 0.81 per cent present in the original casein. On further investigation, however, it became evident that even this dilute alkali had induced a partial hydrolysis of the casein and that the final product was probably not a definite compound. If this were true then, under the more drastic alkaline treatment of Uhl's experiments, he was dealing with racemized and perhaps almost completely hydrolyzed casein split-products on the free amino groups, to which it is possible that carbon disulfide had added in dithiocarbamate linkages. Accordingly Uhl's product probably represents a mixture of various products and should not be classed as a casein "compound."

References

1. Abderhalden, E., *Naturwissenschaften*, **12**, 716 (1924); *Z. physiol. Chem.*, **128**, 119 (1923); **129**, 106, 143 (1923); **130**, 205 (1923); **131**, 284 (1923); **132**, 1 (1924); **133**, 238 (1924); **134**, 113 (1924); **135**, 199 (1924); **139**, 169 (1924); **140**, 92, 99 (1924)
2. Abderhalden, E. and Kempe, M., *Z. physiol. Chem.*, **52**, 212 (1907)
3. Abderhalden, E. and Reich, F., *Z. physiol. Chem.*, **193**, 198 (1930)
4. Abderhalden, E. and Schittenhelm, A., *Z. physiol. Chem.*, **47**, 458 (1906)
5. Abderhalden, E. and Sickel, H., *Z. physiol. Chem.*, **138**, 108 (1924)
6. Abderhalden, E. and Sickel, H., *Z. physiol. Chem.*, **144**, 80 (1925)
7. Alexander, F., *Z. physiol. Chem.*, **25**, 411 (1898)
8. Benedicenti, A., *Arch. Anat. u. Physiol., Physiol. Abt.*, **1697**, 219
9. Bissegger, W., "Weitere Beitrage zur Kenntniss der Stickstoffhaltigen Bestandteil inbesondere der Eiweisskörper des Enmentaler Kases," Inaug. Diss. Zurich (1907)
10. Blum, F., *Z. physiol. Chem.*, **22**, 127 (1896)
11. Bondyznski, St. and Zoja, L., *Z. physiol. Chem.*, **19**, 225 (1894)
12. Bopp, F., *Ann.*, **69**, 16(1849)
13. Braconnot, H., *Ann. Chim. et Phys.*, **13**, 113 (1820)
14. Buraczewski, J. and Krauze, L., *Z. physiol. Chem.*, **71**, 153 (1911)
15. Burn, J. H., *Biochem. J.*, **8**, 154 (1914)
16. Burow, R., "Beiträge zur Entscheidung der Frage ob die Kaseine verscheidener Tierarten identisch sind," Inaug. Diss. Basel (1905)
17. Cherbuliez, E. and De Mandrot, G., *Helv. Chim. Acta*, **14**, 163 (1931)
18. Cohn, E. J., Hendry, J. L. and Prentiss, A. M., *J. Biol. Chem.*, **63**, 721 (1925)

19. Cramer, E., *J. prakt. Chem.*, (1) **96**, 76 (1865)
20. Crowther, C. and Raistrick, H., *Biochem. J.*, **10**, 434 (1916)
21. Dakin, H. D., *J. Biol. Chem.*, **13**, 357 (1912-13)
22. Dakin, H. D., *Biochem. J.*, **12**, 290 (1918)
23. Dakin, H. D. and Dudley, H. W., *J. Biol. Chem.*, **15**, 263 (1913)
24. Dakin, H. D. and Dudley, H. W., *J. Biol. Chem.*, **15**, 271 (1913)
25. Dietrich, M., *Biochem. Z.*, **22**, 120 (1909)
26. Drechsel, *Ber. d. math.-phys. Klasse d. Kgl. Sachs Gesellsch. d. Wiss.*, **44**, 115 (1892), cited by Hoppe, Seyler, Thierfelder in "Handbuch der physiologisch- und pathologisch-chemischen Analyse" 9th ed. p. 257
27. Dudley, H. W. and Woodman, H. E., *Biochem. J.*, **9**, 97 (1915)
28. Dunn, M. S., *J. Am. Chem. Soc.*, **47**, 2564 (1925)
29. Dunn, M. S. and Lewis, H. B., *J. Biol. Chem.*, **49**, 327 (1921)
30. Dunn, M. S. and Lewis, H. B., *J. Biol. Chem.*, **49**, 343 (1921)
31. Edlbacher, S., *Z. physiol. Chem.*, **107**, 52 (1919)
32. Fischer, E., "Untersuchungen über Aminosäuren, Polypeptide und Proteine," Berlin (1906) (A reprint of Fischer's many papers on protein research. Attention is directed particularly to the introduction.)
33. Fischer, E., *Z. physiol. Chem.*, **39**, 155 (1903)
34. Fischer, E. and Abderhalden, E., *Z. physiol. Chem.*, **42**, 540 (1904)
35. Fodor, A. and Kuk, S., *Biochem. Z.*, **240**, 123 (1931) ; **245**, 350 (1932) ; **259**, 331 (1933) ; *Kolloid-Z.*, **74**, 66 (1936)
36. Folin, O., and Looney, J. M., *J. Biol. Chem.*, **51**, 421 (1922)
37. Foreman, F. W., *Biochem. Z.*, **56**, 1 (1913)
38. Foreman, F. W., *Biochem. J.*, **13**, 378 (1919)
39. Fränkel, S. and Friedmann, M., *Biochem. Z.*, **182**, 434 (1927)
40. Freeman, M. E., "The Reaction between Amino Acids and Carbon Disulfide," Thesis, Univ. of Minnesota (1929)
41. von Furth, O., "Einwirkung von Salpetersäure auf Eiweissstoff," Habilitationschrift, Strassburg (1899)
42. Fürth, O. and Deutschberger, O., *Biochem. Z.*, **186**, 139 (1927)
43. Fürth, O. and Minnibeck, H., *Biochem. Z.*, **250**, 18 (1932)
44. Geake, A., *Biochem. J.*, **8**, 30 (1914)
45. Geake, A. and Nierenstein, M., *Biochem. J.*, **8**, 287 (1914)
46. Gortner, R. A. *et al.*, *J. Biol. Chem.*, **26**, 177 (1916) ; *J. Am. Chem. Soc.*, **37**, 1630 (1915) ; **39**, 2477 (1917) ; **42**, 632, 821, 2378 (1920) ; **45**, 550 (1923) ; **46**, 1224 (1924)
47. Gortner, R. A. and Holm, G. E., *J. Am. Chem. Soc.*, **39**, 2736 (1917)
48. Gortner, R. A. and Sandstrom, W. M., *J. Am. Chem. Soc.*, **47**, 1663 (1925)
49. Habermann, J. and Ehrenfeld, R., *Z. physiol. Chem.*, **32**, 467 (1901)
50. Hammarsten, O., *Z. physiol. Chem.*, **7**, 227 (1883)
51. Hansen, A. and Nagel, R., *Z. physiol. Chem.*, **196**, 201 (1931)
52. Hart, E., *Z. physiol. Chem.*, **33**, 347 (1901)
53. Hempel, W., *Arch. ges. Physiol.*, **56**, 558 (1894)
54. Herzig, J. and Landsteiner, K., *Biochem. Z.*, **61**, 458 (1914)
55. Hinterberger, F., *Ann.*, **71**, 70 (1849)
56. Hoffman, W. F. and Gortner, R. A., "Colloid Symposium Monograph," **2**, 209, New York, Reinhold Publishing Corp., 1925
57. Hoffman, W. F. and Gortner, R. A., *J. Phys. Chem.*, **29**, 769 (1925)
58. Holter, H., Linderstrøm-Lang, K. and Funder, J. B., *Compt. rend. Lab. Carlsberg*, **19**, No. 10, 1 (1933)
59. Hopkins, F. G. and Pinkus, S. N., *Ber.*, **31**, 1311 (1898)
60. Inouye, K., *Z. physiol. Chem.*, **81**, 80 (1912)
61. Johnson, T. B., *J. Am. Chem. Soc.*, **37**, 2598 (1915)

62. Levene, P. A. and Bass, L. W., *J. Biol. Chem.*, **78**, 145 (1928); **82**, 171 (1929)
63. Levene, P. A. and Hill, D. W., *J. Biol. Chem.*, **101**, 71, 711 (1933)
64. Levene, P. A. and Van Slyke, D. D., *J. Biol. Chem.*, **6**, 419 (1909)
65. Levene, P. A. and van der Scheer, J., *J. Biol. Chem.*, **22**, 425 (1915)
66. Levites, S. J., *Z.physiol. Chem.*, **43**, 202 (1904); *Biochem. Z.*, **20**, 224 (1909)
67. Lewis, H. B. and Updegraff, H., *J. Biol. Chem.*, **56**, 405 (1923)
68. Lieben, F. and Bauminger, B., *Biochem. Z.*, **261**, 374 (1933)
69. Liebig, J., *Ann.*, **57**, 127 (1846)
70. Liebrecht, A., *Ber.*, **30**, 1824 (1897)
71. Lipmann, F., *Biochem. Z.*, **262**, 3, 9 (1933); *Naturwissenschaften*, **21**, 236 (1933)
72. Malfatti, H., *Z. physiol. Chem.*, **16**, 69 (1892)
73. Mörner, K. A. H., *Z. physiol. Chem.*, **34**, 285 (1901-2)
74. Mueller, J. H., *J. Biol. Chem.*, **56**, 157 (1923)
75. Okuda, Y., *J. Dept. Agr. Kyushu Imp. Univ.*, **1**, 163 (1925)
76. Osborne, T. B. and Harris, I. F., *J. Am. Chem. Soc.*, **25**, 323 (1903)
77. Osborne, T. B., Leavenworth, C. S. and Brautlecht, C. A., *Amer. J. Physiol.*, **23**, 180 (1908)
78. Palmer, L. S. and Richardson, G. A., "Colloid Symposium Monograph," **3**, 112, New York, Reinhold Publishing Corp., 1925
79. Panzer, T., *Z. physiol. Chem.*, **33**, 131 (1901)
80. Panzer, T., *Z. physiol. Chem.*, **34**, 66 (1901-2)
81. Pirie, N. W., *Biochem. J.*, **26**, 1270 (1932)
82. Plimmer, R. H. A. and Bayliss, W. M., *J. Physiol.*, **33**, 439 (1905-6)
83. Posternak, S., *Compt. rend. Acad. Sci.*, **184**, 306 (1927)
84. Posternak, S. and Posternak, T., *Compt. rend. Acad. Sci.*, **187**, 313 (1928)
85. Proust, M., *Ann. Chim. et Phys.*, **10**, 29 (1818)
86. Reh, A., *Beitr. chem. Physiol. u. Path.*, **11**, 1 (1908)
87. Rimington, C., *Biochem. J.*, **21**, 1179 (1927)
88. Ringer, M. and Underhill, F. P., *J. Biol. Chem.*, **48**, 503 (1921)
89. Ritthausen, H., *J. prakt. Chem.*, **103**, 193 (1868); **106**, 445 (1869); **107**, 218 (1869)
90. Robertson, T. B., "The Physical Chemistry of the Proteins," New York, Longmans Green & Co., 1918
91. Salkowski, E., *Arch. ges. Physiol. (Pflüger's)*, **63**, 401 (1896); *Z. physiol. Chem.*, **27**, 297 (1899); **32**, 245 (1901)
92. Salkowski, E., *Biochem. Z.*, **136**, 169 (1923)
93. Salkowski, E. and Hahn, M., *Arch. ges. Physiol. (Pflüger's)*, **59**, 225 (1895)
94. Sandstrom, W. M., *J. Phys. Chem.*, **34**, 1071 (1930)
95. Schmidt, G., *Proc. 14th Internat. Congr. Physiol.*, **228**, see *Z. physiol. Chem.*, **223**, 86 (1933)
96. Schuberthowna, M., *Bull. Intern. Acad. Sci. Cracovic* (A), **1912**, 705, see *Chem. Abstr.*, **7**, 491 (1913) (Original not seen)
97. Schwarz, L., *Z. physiol. Chem.*, **31**, 460 (1900)
98. Siegfred, M., *Z. physiol. Chem.*, **43**, 46 (1904); **50**, 163 (1906-7)
99. Skraup, Z. H., *Monatsch. f. Chem.*, **25**, 633 (1904); **26**, 1343 (1905)
100. Skraup, Z. H. and Hoernes, P., *Monatsch. f. Chem.*, **27**, 631 (1906)
101. Skraup, Z. H. and Krause, E., *Monatsch. f. Chem.*, **30**, 447 (1909)
102. Skraup, Z. H. and Witt, R., *Monatsch. f. Chem.*, **27**, 663 (1906)
103. Sørensen, M. and Haugaard, G., *Biochem. Z.*, **260**, 247 (1933)
104. Sullivan, M. X., *U. S. Pub. Health Reports* No. 78 (1929)
105. Svedberg, T., Carpenter, L. M. and Carpenter, D. C., *J. Am. Chem. Soc.*, **52**, 241 (1930); **52**, 701 (1930)
106. Tangl, F., *Arch. ges. Physiol. (Pflüger's)*, **121**, 534 (1908)

107. Tillmans, J. and Philippi, K., *Biochem. Z.,* **115,** 36 (1929)
108. Treves, Z. and Salomone, G., *Biochem. Z.,* **7,** 11 (1907)
109. Trillat, A., *Compt. rend. Akad. Sci.,* **114,** 1278 (1892)
110. Troensegaard, N. and Mygind, H. G., *Z. physiol. Chem.,* **184,** 147 (1929); **193,** 171 (1930); **199,** 133 (1931)
111. Uhl, R., *Z. physiol. Chem.,* **84,** 478 (1913)
112. Underhill, F. P., Greenberg, P. and Alu, A. F., *J. Biol. Chem.,* **48,** 549 (1921)
113. Underhill, F. P. and Hendrix, B. M., *J. Biol. Chem.,* **22,** 465 (1915)
114. Underhill, F. P. and Hjort, A. M., *J. Pharm. Exptl. Therap.,* **19,** 145 (1922)
115. Underhill, F. P. and Ringer, M., *J. Pharm. Exptl. Therap.,* **19,** 179 (1922)
116. Vandevelde, A. J. J., *Rec. trav. chim. Pays-Bas.,* **44,** 900 (1925); *Bull. acad. roy. med. Belg.,* (5) **5,** 473 (1925)
117. Vandevelde, A. J. J., *Rec. trav. chim. Pays-Bas.,* **45,** 825 (1926); *Bull. acad. roy. med. Belg.,* (5) **9,** 408 (1929)
118. Van Slyke, D. D., *Ber.,* **43,** 3170 (1910)
119. Van Slyke, D. D., *J. Biol. Chem.,* **10,** 15 (1911)
120. Van Slyke, D. D., *J. Biol. Chem.,* **16,** 531 (1914)
121. Van Slyke, L. L. and Bosworth, A. W., *J. Biol. Chem.,* **14,** 203 (1913); see also Bosworth, *ibid.,* **15,** 230 (1913); **19,** 397 (1914)
122. Vaughan, V. C., Vaughan, V. C., Jr., and Vaughan, J. W., "Protein Split Products in Relation to Immunity and Disease," Philadelphia and New York, Lea and Febiger, 1913
123. Wada, M., *Biochem. Z.,* **257,** 1 (1933)
124. Wells, H. G., "The Chemical Aspects of Immunity," p. 73, New York, Chemical Catalog Co. (Reinhold Publishing Corp.), 1925
125. Weyl, Th., *Ber.,* **21,** 1407 (1888); **21,** 1529 (1888)
126. Wiley, F. H. and Lewis, H. B., *J. Biol. Chem.,* **86,** 511 (1930)
127. Willstätter, R., *Ber.,* **35,** 1378 (1902)
128. Wright, N. C., *Biochem. J.,* **18,** 245 (1924)
129. Wroblewski, A., "Beiträge zur Kenntniss des Frauencaseins und seiner Unterscheide vom Kuhcasein," Inaug. Diss. Bern. (1894)

Additional References

130. Abderhalden, E., *Z. physiol. Chem.,* **44,** 23 (1905)
131. Bancroft, W. D. and Browning, H. F., *J. Phys. Chem.,* **41,** 1163 (1937)
132. Barger, G. and Coyne, F. P., *Biochem. J.,* **22,** 1417 (1928)
133. Bosworth, A. W. and Van Slyke, L. L., *J. Biol. Chem.,* **19,** 67 (1914); *Tech. Bull. 37, N. Y. (Geneva) Agr. Expt. Sta.*
134. Csonka, F. A. and Horn, M. J., *J. Biol. Chem.,* **93,** 677 (1931)
135. Groh, C. and Weltner, M., *Z. physiol. Chem.,* **198,** 267 (1931)
136. Hanke, M. T., *J. Biol. Chem.,* **66,** 489 (1925)
137. Herzig, J. and Lieb, H., *Z. physiol. Chem.,* **117,** 1 (1921)
138. Jones, D. B. and Gersdorff, C. E. F., *J. Biol. Chem.,* **104,** 99 (1934)
139. Kapeller-Adler, R., *Biochem. Z.,* **252,** 185 (1932)
140. Kimura, H., *Acta Schol. Med. Univ. Imp. Kyoto,* **11,** 117 (1928)
141. Kober, P. A., *J. Biol. Chem.,* **22,** 433 (1915)
142. Leavenworth, C. S., *J. Biol. Chem.,* **61,** 315 (1924)
143. Lepinois, E., *J. pharm. Chem.,* (6) **5,** 561 (1897)
144. Lieben, F. and Müller, R., *Biochem. Z.,* **197,** 119 (1928)
145. Lieben, F. and Lazzlo, D., *Biochem. Z.,* **159,** 110 (1925)
146. Lieben, F., *Biochem. Z.,* **147,** 174 (1924)
147. Masui, S., *Acta Schol. Med. Univ. Imp. Kyoto,* **13,** 264 (1931)
148. Nakashima, F., *J. Biochem.,* (Japan) **5,** 293 (1925)
149. Osborne, T. B. and Guest, H. H., *J. Biol. Chem.,* **9,** 352 (1911)
150. Pellizza, A., *Bull. soc. ind. Mulhouse,* **85,** 295 (1919)

151. Sadikov, V. S. and Poshiltzova, E. A., *Biochem. Z.* **221**, 304 (1930); *Shur Prikladnoi Khim.*, **3**, 895 (1930)
152. Steudel, H. and Schumann, R., *Z. physiol. Chem.*, **183**, 168 (1929); Steudel, *ibid.*, **187**, 203 (1930); Steudel and Wohinz, R., *ibid.*, **196**, 78 (1931)
153. Teruuchi, Y. and Okabe, L., *J. Biochem.* (Japan), **8**, 459 (1928)
154. Vickery, H. B. and White, A., *J. Biol. Chem.*, **99**, 706 (1933)
155. Vickery, H. B. and White, A., *J. Biol. Chem.*, **103**, 413 (1933)
156. White, A., *J. Biol. Chem.*, **103**, 295 (1933)
157. Yaichnikov, I. S., *J. Gen. Chem.*, (U.S.S.R.) **3**, 1 (1933)
158. Yaichnikov, I. S., *J. Gen. Chem.*, (U.S.S.R.) **4**, 1114 (1934)

Chapter 3

The Physical Chemistry of Casein

FREDERICK L. BROWNE

Satisfactory treatment of the physical chemistry of casein at the present time is difficult because fundamental conceptions of the nature of casein have changed in recent years. Casein as ordinarily prepared is no longer considered a single chemical substance but rather a mixture, the exact nature of which is not yet understood. Moreover the molecules or micelles of casein are now known to be very much larger than was formerly supposed, a fact that renders analogies with the physical chemistry of simpler substances less tenable. Most of the literature on the physical chemistry of casein needs reinterpretation in the light of the findings of the last dozen years, but the newer developments have not yet shaped themselves definitely enough to make reinterpretation practicable. Some scientists apparently feel that the older literature should be disregarded, but the writer is unable to agree with that opinion. To the technologist the older literature will remain for some time a source of useful empirical knowledge and therefore it cannot be ignored even though some of its theoretical background must be viewed with much skepticism.

The Heterogeneity of Casein

In nearly all the older literature casein was assumed to be a homogeneous chemical substance.[81, 162, 165, 179, ch.2] Its composite nature escaped detection so long because there were no convenient criteria of chemical purity applicable to it. As a solid it is amorphous (a claim for its crystallization[158] is very doubtful) and it decomposes before a melting point is reached. Its solubilities are ambiguous and its solutions do not lend themselves well to measurement of colligative properties. Its compounds are not sharply defined and have not yielded readily to interpretation by means of the phase rule. Its chemical individuality was accepted chiefly because it hangs together through a reasonably wide variety of processes, and had never separated into components differing significantly in composition and properties except under circumstances under which chemical decomposition might be expected.

Fractionation by Differences in Solubility

In 1918 Osborne and Wakeman[149] found small proportions of a protein differing distinctly from casein in the alcoholic washings from recently precipitated casein. The new protein was regarded merely as a foreign contaminant against which precautions should be taken in preparing pure casein. Not until 1925 was the homogeneity of casein seriously questioned. It was then found that, when an excess of casein is added to a dilute solution of hydrochloric acid, less casein dissolves, the greater the excess left undissolved.[110] Adsorption or reaction of acid with the undissolved excess of casein accounted for that trend; but when the concentrations of hydrogen and chloride ions in the solution were kept constant by properly proportioning the quantities of hydrochloric acid and sodium chloride to the quantity of casein added, the solubility was greater, the greater the excess of casein undissolved.[124] Similarly, in solutions of sodium hydroxide and sodium chloride at pH 5 to 5.5 the solubility of casein increased with the undissolved excess.[197] By extracting casein with dilute hydrochloric acid or by dissolving it either in hydrochloric acid or in sodium hydroxide and then precipitating part of it by partially neutralizing the solvent, casein was separated into fractions that differed not only in solubility but in precipitability by calcium salts, and also in percentage content of nitrogen and phosphorus and in ratio of nitrogen to phosphorus.[125]

Since an increase in solubility with excess of solute is characteristic of the peptization of numerous colloids both organic and inorganic,[151] it was suggested[25, 26] that the facts proved merely that casein forms colloidal dispersions rather than true solutions, and that dry casein is a gel containing fractions differing in structure and therefore in solubility. The purely colloidal point of view, however, failed to account for the separation of casein into fractions of distinctly different chemical composition and permanently altered solubility.[197] Both the dependence of solubility on the excess remaining undissolved and the separation into fractions differing in composition are in accord with the theory that casein is a complex of several reactive substances bound together firmly enough to pass unchanged through the processes to which casein is ordinarily subjected, but capable of undergoing reversible dissociation when in contact with solvents. This theory had previously been advanced by Sørensen[193, 194] to account for similar peculiarities in the solubility of the serum globulins.

In an exhaustive study Linderstrøm-Lang[123] prepared a series of seven fractions by extracting casein with 60-per cent alcohol made slightly acid with hydrochloric acid and precipitating the extracts with sodium hydroxide. The three major fractions amounted to 54.2, 20.2, and 14.3 per cent, the others to 5.5, 3.0, 1.7, and 0.6 per cent of the

original casein respectively. The fractions differed significantly in content of phosphorus, ratio of phosphorus to nitrogen, content of arginine, histidine, tyrosine, and tryptophane, solubility, specific rotation of the solutions, precipitability with calcium chloride, time of coagulation with rennet, combining equivalent for hydrochloric acid, rate of digestion by enzymes, and formol titration. When the fractions were mixed again, a product having the same properties as the original casein was obtained.

Other methods of separating casein into fractions were found by subsequent investigators. It was accomplished by extraction with aqueous solutions of neutral salts such as magnesium sulfate, sodium chloride, ammonium sulfate, ammonium chloride,[32] sodium benzenesulfonate and sodium cymenesulfonate.[141a] It was effected also by addition of absolute ethyl alcohol to a solution of casein in 40 per cent urea or to a solution of casein in anhydrous phenol at 70°C., and by addition of hydrochloric acid to a solution of casein in ammonium hydroxide containing 70 per cent ethyl alcohol;[77] the corresponding fractions obtained by each of these three methods appeared to be identical and when the fractions obtained by any one method were mixed the properties of the original casein were restored.

Cherbuliez and Meyer[31] obtained four fractions by precipitation from alkaline ammonium chloride solution by alternate addition of hydrochloric acid and acetone. Each of the fractions appeared to be chemically homogeneous upon analysis for content of amino acids. In different samples of casein, however, the four fractions occurred in widely different proportions. Only one of them had the property of coagulating to a firm curd with rennet, and the suitability of the milk of different cows for making cheese ran parallel to the content of the coagulable fraction in the casein.[30]

Linderstrøm-Lang[123] did not regard his fractions as pure chemical individuals completely freed from one another although he considered complete separation practicable. Subsequent investigators believed one or more of their fractions to be homogenous.[31, 100] Carpenter and Hucker,[29] by extraction with acid 70-per cent alcohol and fractionation with potassium oxalate, obtained three products with homogeneous micellar weights of 98,000, 188,000, and 375,000 respectively which were clearly distinguishable from one another and from the alcohol-soluble protein of Osborne and Wakeman by means of serologic reactions. Demanez,[43] using fractions made by Linderstrøm-Lang's method, was unable to differentiate them by precipitin reactions in casein antiserum from a cow, although such methods distinguish casein from paracasein[148] and casein of cow, sheep, and goat milk from that of human and mare milk.[44]

Fractionation by Ultracentrifugal Sedimentation

The clearest evidence of the heterogeneity of casein comes from studies of the size and weight of the micelles in solutions of proteins by sedimentation in the ultracentrifuge.[203]

Svedberg[204] found that most naturally occurring proteins are monodisperse, that is, have micelles of uniform size, but that commercial preparations of proteins are often polydisperse because they have been partly decomposed by clumsy methods of isolation. The pure, monodisperse proteins, moreover, generally have micellar weights of 34,500 or a multiple of 34,500. The micelle is a strictly physical conception of small particles capable of independent motion in the medium in which they are dispersed and large enough to be separated from the liquid medium by an interface that is a seat of interfacial energy. The molecule, on the other hand, is a chemical conception of minimum units capable of exhibiting the chemical reactions characteristic of the substance. In typical true solutions the molecules, or the ions into which the molecules dissociate, are free to move independently and are therefore "monodisperse," but in size they are too small to be considered colloidal micelles. On the other hand typical colloids whose study led to the concept of micelles are usually polydisperse, the micelles being aggregates of varying numbers of molecules. It was therefore reasonable to assume that the micelles in monodisperse proteins are single large molecules or macromolecules[165a] and that polydispersity in protein solutions indicates chemical heterogeneity either because the protein is naturally a mixture or because it is partially decomposed during its isolation.

In milk Svedberg[205] found that casein is polydisperse, its micelles ranging from 20 to 140 mμ in diameter. The casein in milk, however, is not a "true protein solution" but a "suspension of the calcium salt of casein that is difficultly soluble at the pH of milk." Casein was therefore prepared carefully by several methods, dissolved in phosphate buffer solutions at pH 6.8, and examined in the ultracentrifuge.[207] All the preparations proved to be mixtures of micelles of widely different weights, the proportions of which varied even in different preparations of casein made by the same method. In Van Slyke and Baker casein most of the material had a micellar weight of 75,000 to 100,000 if dissolved at room temperature; but if dissolved at 40°C. most of it had a weight of 188,000. A product extracted from Hammarsten casein with hot acidified 70 per cent alcohol and amounting to about one-third of the sample was monodisperse with a micellar weight of 375,000.

Earlier investigators,[15, 211] basing their estimates largely on the content of sulfur and phosphorus in casein, the combining capacity for

bases, and the assumptions of homogeneity and monodispersity, calculated the molecular weight of casein to be roughly 8,800 and that of paracasein 4,400. As the nature and proportions of the amino acids in casein became more exactly known it was realized that, if casein were homogeneous, the molecular weight would have to be much larger to admit a minimum of one molecule of each amino acid in each molecule of casein. Careful review of the evidence in 1925 led Cohn, Hendry and Prentiss[39] to estimate 192,000 for the minimum molecular weight of casein. Cryscopic measurements of solutions of casein in phenol seemed to be consistent with that value[37] but subsequent measurements of osmotic pressure of solutions in phenol[11] and in 6.66*M* aqueous urea[24] led to the much smaller values of 25,000 and 33,600 respectively. Apparently the micelles in which casein is dispersed under some circumstances are smaller than the minimum molecule consistent with the assumption that it is a homogeneous substance.

Physically casein is clearly heterogeneous both in milk and in the solutions ordinarily made from it. That casein is likewise chemically heterogeneous, is evident from the facts that it is separable into fractions of distinctly different chemical composition by methods in which it is kept at all times in a region of pH very close to its isoelectric point, and that on mixing the fractions a product having the properties of the original casein is obtained. The ease with which casein is seriously altered both physically and chemically merely by heating its solutions or suspensions, and restored to its original condition by dissolving and reprecipitating strongly supports Sørensen's view that it is a complex of reactive ingredients that readily undergo association and dissociation. It has in fact been suggested that all the proteins of milk may be regarded merely as varying stages of aggregation or combination of the same group of elementary substances.[46, 126] At any rate the casein separated from milk, which is the product in which the technologist is interested, is a mixture, and a mixture that is very easily altered in nature.

Solubility

In spite of extensive literature on the subject, the solubility of casein must be discussed for the most part in qualitative terms. Data reported in quantitative terms should be regarded as illustrative of trends and general orders of magnitude.

The sharp separation of solid and liquid phases usually possible with simple crystalline solutes can rarely be effected with casein. Dry casein is essentially a granulated jelly, the granules of which imbibe solvent and swell markedly before passing into solution, and the clots precipitating from solution may enclose solvent and possess a structure

differing seriously from that of the swollen granules. Establishment of a solubility equilibrium by approach from both sides is therefore difficult or impracticable.[124, 125] Moreover the procedure followed in separation and drying profoundly affects the solubility of casein, so that best results are obtained by using absolutely freshly precipitated casein while it is still in contact with water.[197] Experiments are reported[25, 26] in which a Hammarsten casein was made so difficultly soluble by heating for 180 hours at 90 to 92°C. that it was insoluble in sodium hydroxide weaker than 0.2*N* and only partially soluble in 0.5*N* sodium hydroxide. In general the solubility is decreased the lower the moisture content to which casein is dried, the higher the temperature at which it is dried, by contact with alcohol or ether, and on aging.[107, 161, 187] Decrease in solubility on aging is faster the greater the moisture content of the casein while it is stored.[58]

Casein, as would be expected from its derivation from amino acids, is amphoteric in nature, *i.e.,* capable of combination either with acids or bases. At the isoelectric point, pH 4.6, it combines with neither acids nor bases; below pH 4.6 it combines with acids and above pH 4.6 it combines with bases.[78, 128, ch. 1] The salts formed are electrolytic in character; in aqueous solution the salts dissociate into ions, the casein appearing in the cation on the acid side of the isoelectric point and in the anion on the alkaline side. The solubility of casein is minimum at the isoelectric point and increases rapidly on either side; but the characteristics of the solutions on the two sides of the isoelectric point are distinctly different. For these reasons the principal measuring stick in surveying the properties of aqueous solutions of casein should be the scale of pH.

The older theory of the ionization of amino acids and proteins and their reactions with acids and bases, which will probably prove more familiar to most of the readers of this book, may be expressed:

$$\begin{array}{ccc} NH_2.R.COOH + H_2O \rightleftharpoons HO.NH_3.R.COOH & & HO.NH_3.R\ COOH \\ + & \text{or} & + \\ HCl \rightleftharpoons H^+ + Cl^- & & NaOH \rightleftharpoons Na^+ + OH^- \\ \downarrow\uparrow & & \downarrow\uparrow \\ H_2O + Cl^- + {}^+NH_3.R.COOH & & HO.NH_3.R.COO^- + Na^+ + H_2O \end{array}$$

Ionization of the amino acid is minimum at the isoelectric point. Acids react with the amino group and bases with the carboxyl group. The salts formed are much more highly ionized than the isoelectric amino acid. A newer theory of amphoteric ions ("zwitterion" theory)[233, 237, p. 700] may be expressed:

$$\begin{array}{ccc} HO.NH_3.R.COOH \rightleftharpoons {}^+NH_3.R.COO^- + H^+ + OH^- & & H^+ + OH^- + {}^+NH_3.R.COO^- \\ + & \text{or} & + \\ HCl \rightleftharpoons Cl^- + H^+ & & NaOH \rightleftharpoons OH^- + Na^+ \\ \downarrow\uparrow & & \downarrow\uparrow \\ Cl^- + {}^+NH_3.R.COOH + H_2O & & H_2O + HO.NH_3.R.COO^- + Na^+ \end{array}$$

In contrast to the older theory, the theory of amphoteric ions postulates maximum ionization at the isoelectric point. The amphoteric ions do not conduct electricity because their net charge is zero but, since they are strongly polarized, their presence is revealed by their effect on the dielectric constant of the solution. Acids react with the carboxyl group by repressing its ionization, and bases similarly react with the amino group. The salts formed are less highly ionized than the isoelectric protein. In both theories the casein is the cation in casein chloride and the anion in sodium caseinate. Although the two theories describe ionization and neutralization in diametrically opposite terms they actually paint essentially the same picture except for phenomena that depend upon ionic polarization. The older theory is followed in the remainder of this book.

Solubility Near the Isoelectric Point

At its isoelectric point casein is commonly said to be insoluble in water.[120, 134, 150, 171] It is soluble, however, to the extent of 0.05 gram per liter at 5°C.[159] and 0.02 to 0.11 gram at 25°C.[34, 102] When dry casein is brought into contact with distilled water the pH comes to 4.9[34] rather than 4.6. Apparently the isoelectric point* can be shifted slightly under different circumstances because neutral salts such as sodium chloride may move it nearly to pH 4.0[112, 197] while lanthanum chloride moves it above pH 4.6.[143] Paracasein has practically the same solubility as casein.[160]

Casein is more soluble in dilute solutions of neutral salts than in pure water, a fact that is in accord with a general tendency among slightly soluble substances.[35] As the concentration of salt increases, the solubility usually reaches a maximum after which it falls off again, possibly because caseinates are formed whose solubility is diminished by the effect of a common ion in repressing ionization of the caseinate.[35] The maximum solubility of casein, in grams per liter, is 7.6 in 0.5*N* sodium chloride, 4.7 in 0.5*N* potassium chloride, 5.5 in 1*N* calcium chloride, 3.6 in 0.2*N* barium chloride, 3.8 in 1*N* strontium chloride, 6.5 in 1*N* magnesium chloride, 3.3 in 0.01*N* potassium sulfate, 4.4

* There are two ways of defining the isoelectric point, corresponding to two levels of electric potential between a micelle and the solution in which it is dispersed. The total potential, ϵ, determines solubility and reactivity and in the case of casein is zero at the fixed pH 4.6. In the solution about the micelle there is an "atmosphere" of ions of opposite charge drawn electrostatically to the micelle and through this atmosphere the potential falls off gradually. When the micelle moves, as in cataphoresis, it carries an adhering envelope of solution with it, and the potential governing the rate of movement is not ϵ but the electrokinetic potential, ζ, at the surface of the aqueous envelope, which depends both on ϵ and on the ionic atmosphere. The ζ potential therefore depends on the kind and amount of electrolyte in the solution and may be zero when ϵ is still finite.[202]

in 0.1N magnesium sulfate, 15.1 in 0.5N sodium fluoride, and 3.8 in 0.15N disodium phosphate.[208, p.20] The maximum solubility in sodium chloride is given by another investigator[182] as 3.46 grams per liter at 0.115N, but such discrepancies may be expected because of the many factors that may alter the solubility of casein. For example Sørensen[196] found the solubility in 0.05N sodium chloride at pH 5.20 to be 0.8 gram per liter when 2.19 grams of casein were added, and 6.55 grams per liter when 21 grams were added to the same amount of solution.

McKee and Gould[141a] recently reviewed the literature on the solubility of casein in aqueous solutions of salts having a pH of 4.6 and reported data of their own. In 5 per cent sodium cymenesulfonate solution the solubility of casein in grams per liter of solvent is 30.4 at 15°, 59.4 at 30°, and 96.8 at 45°C., while in 7.5 per cent solution it is 53.0 at 15° and 80.2 at 30°C. In 5.0 per cent sodium benzenesulfonate the solubility is 9.8 at 15°, 19.3 at 30° and 30.5 at 45°C., while in 10 per cent solution it is 22.1 at 15°, 27.5 at 30°, and 48.2 at 45°C. In 2.5 per cent potassium thiocyanate the solubility is 17.9 at 15°, 25.1 at 30° and 34.2 at 45°C., while in 5.0 per cent solution it is 35.5 at 15°C.

Concentrated aqueous solutions of very soluble, highly hydrated salts, such as lithium thiocyanate, calcium thiocyanate, calcium iodide, and calcium bromide dissolve casein readily at the boiling temperature and the concentrated solutions of casein remain stable when cooled. Lithium thiocyanate may dissolve casein at 25°C. It is also possible with these salts to convert casein into the "ropy-plastic" condition in which it can be spun in threads and fibers.[216]

As would be expected, the solubility of casein in solutions of salts that are hydrolytically dissociated is greater than its solubility in solutions of neutral salts. In 0.1N solution at 25°C., for example, the solubility in grams per liter is 1.88 in potassium bromide, 4.51 in ammonium nitrate, 4.06 in sodium oxalate, 9.27 in ammonium thiocyanate, 11.28 in sodium propionate, more than 20 in sodium valerate, potassium acetate, and potassium cyanide,[171] and 14 in sodium tartrate.[50] In strongly hydrolyzed salts the solubility does not pass through a maximum with increasing concentration of salt but continues to increase. Concentrated solutions of casein with pH between 4.6 and 7.0 can be made by swelling the granules of casein greatly in aqueous solutions of suitable salts, such as sodium fluoride, and then dissolving it with hydrolyzed salts such as borax.[5]

Solubility in Nonaqueous and Mixed Solvents

Casein is insoluble in most of the common organic solvents but it is soluble in alcohol containing either hydrochloric acid or sodium hydroxide.[9] There are a few anhydrous solvents in which it is materially soluble. Casein swells and dissolves in anhydrous formic acid,[71, 185, 177]

in mixtures of formic and acetic acid though not in glacial acetic acid alone,[135] in 85 per cent glacial lactic acid,[71, 135] in glacial phosphoric acid, in pyruvic acid,[135] and in concentrated phenol.[37, 77] The solutions in formic acid are electrolytically conducting, the casein having an equivalent conductance of 35 to 42 mhos, but in lactic acid the low conductance of the solvent is not increased by solution of casein.[71] The solutions in these solvents are much less viscous and lack the turbidity of aqueous solutions of casein and are not precipitated by the usual precipitants for aqueous solutions. Solutions in warm formic acid containing 10 per cent or more of casein set to jellies on cooling. Limits of solubility of casein in these solvents are not recorded, but solutions are reported containing 28 grams per liter in lactic acid and 56 grams per liter in formic acid at room temperature. Addition of water at first changes the clear solutions to turbid, more viscous ones and then precipitates the casein.

Casein is also soluble in a number of mixtures of water and organic solvents which alone are unable to dissolve it appreciably. It is markedly soluble in hot 50 per cent alcohol;[171] and in 50 per cent pyridine at 20 to 25°C. it is soluble to the extent of 5.6 grams per liter.[121] It is readily soluble in 6.66*M* aqueous solutions of urea[24, 77] and in strong aqueous solutions of the dihydroxybenzenes[209] and pyrogallol[217] or chloral hydrate.[209] On diluting these solutions with water, casein precipitates. Casein may also be dissolved in acidified solutions of alcohol in water and in acidified solutions of alcohol in benzene.[50]

Solubility in Aqueous Acids

The difference in solubility between freshly precipitated casein and dried casein is particularly striking in aqueous acids.[179, p. 96] The granules of dried casein swell markedly and soften before passing into solution in acids. Apparently during drying the molecules or micelles of free casein become firmly united in a structure such that each granule may be regarded as a giant micelle, much as a single crystal of sodium chloride may be regarded as a giant molecule. In passing into solution again the crystal of sodium chloride is torn down layer by layer as ions of sodium and chlorine diffuse into the solution, the interior of the crystal meantime remaining practically dry. The granule of casein, on the other hand, imbibes solution, swells and softens. Cohesion of the structure is pitted against pressure of swelling and the granule holds together until the solvent is strong enough to overbalance the cohesion.[134] In a solvent not quite strong enough to effect solution mechanical agitation turns the balance in its favor. Wasting away from the surface of the granules apparently plays a small part until the cohesion is greatly weakened by swelling.

The swelling of casein in acids begins rapidly but proceeds with dimin-

ishing velocity as the final limit of swelling or solution is approached. The velocity is faster at higher temperatures and at higher concentrations of acid[213, 214] up to the optimum concentration at which the pH of the

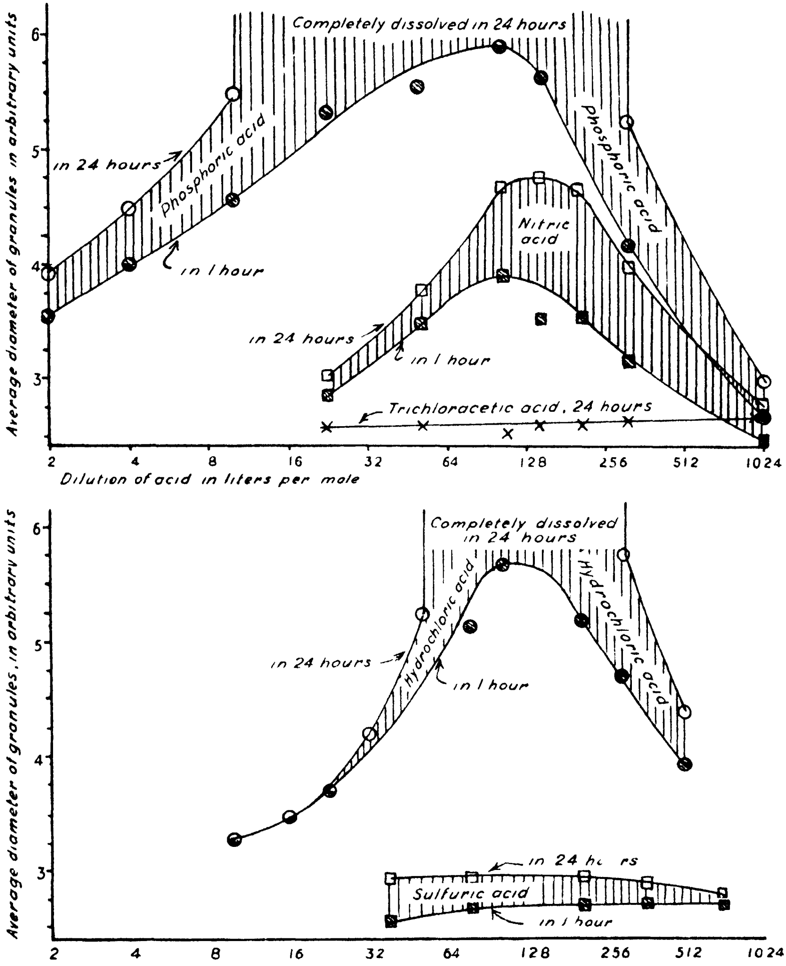


Figure 2.—Swelling and solution of granules of dry casein in varying dilutions (reciprocals of concentration) of phosphoric, nitric, and trichloracetic acids (upper diagram) and hydrochloric and sulfuric acids (lower diagram) after standing for 1 hour and for 24 hours at 24°C.[134]

solution is 2 to 2.3,[145] after which it falls off again. Casein is somewhat more readily soluble in phosphorous, or phosphoric, acid than in hydrochloric acid, less soluble in nitric acid, monochloracetic acid, tartaric acid

and lactic acid, and only slightly soluble in sulfuric acid, acetic acid, oxalic acid, and trichloracetic acid.[50, 56, 134, 213, 214] Organic acids containing a hydroxyl group or a second carboxyl group swell casein more than the corresponding monocarboxyl acids; substitution of chlorine in an organic acid increases, and substitution of an amino group decreases its swelling of casein.[56, 93]

The nature of the relations in aqueous acids is well illustrated by the experiments of Loeb and Loeb[134] on the swelling of granules of dry casein in solutions of five acids at different concentrations, as shown in Fig. 2. A small amount of casein in granules of uniform size, sifted through a 100-mesh but retained on a 120-mesh sieve, was placed in aqueous solutions of acids and the average diameter of the granules carefully measured with a micrometer under a microscope after standing one hour and again after 24 hours at 24°C. The swelling, most of which took place in the first hour, reached a pronounced maximum at approximately 0.01*M* concentration of acid except in sulfuric and trichloracetic acids, which caused little swelling. After 24 hours in phosphoric and hydrochloric acids the granules of casein were entirely dissolved in the regions of concentration corresponding to the gaps in the curves, where the pH of the solutions was between 1.8 and 2.9.

The amount of casein dissolved at 20°C. in hydrochloric acid solutions of varying pH, when one gram of dry casein was put in 100 cubic centimeters of solution, is given in Table 4 from the data of Loeb and Loeb.[134] The solubility, however, varies greatly with the age and previous history of the casein.[110]

TABLE 4.—Solubility of a Sample of Dry Casein in Aqueous Hydrochloric Acid

(*Date of Loeb and Loeb*[134])

pH of the HCl solution	Amount of casein dissolved in 1 hour	22 hours
	grams per liter	
1.40	2.19	3.00
1.87	4.01	7.10
2.06	5.38	7.79
2.18	5.47	7.88
2.36	....	7.33
2.64	3.48	6.34
2.97	2.49	2.67
3.32	0.55	1.33
4.36	0.42	1.02

Addition of inorganic salts even in small percentages to aqueous acids depresses the swelling and solution of casein, and addition of salts to solutions of casein in acids precipitates the casein (see under *Colloidal Behavior*).

Solubility in Aqueous Alkalies

Casein, particularly dry casein, is much more readily soluble and soluble to a greater extent in aqueous alkalies than in aqueous acids. Practically all solutions of casein of technologic importance are effected with aqueous alkalies or strongly hydrolyzed salts of alkaline reaction.

Robertson[173] found that the velocity of solution of casein in alkalies follows none of the laws for solution of crystalline substances in water or in solutions with which they react, but instead follows the equation $x = kt^m$ in which x is the amount of casein dissolved in time t, and k and m are constants for the particular casein and conditions under which solution takes place. Most of the casein goes into solution fairly promptly, but an astonishingly long time is often required for the last traces to dissolve and for equilibrium to be reached.[172] The same law applies to the rate at which casein absorbs acid from dilute solutions in which it is swelling, and to the penetration of water into a column of sand or piece of filter paper. Robertson therefore concluded that the velocity of solution is governed by the rate of penetration of the solution into the granules of casein and that the mechanism of solution in alkalies and in acids is much the same.

Loeb and Loeb,[134] on the other hand, considered the mechanism entirely different in the two media, and that in alkalies granules of casein dissolve without swelling much as do granules of sodium oleate, which lowers the interfacial tension so that projecting small particles are torn off, leaving the granules with smooth surfaces and constantly diminishing size. Detachment of micelles from the surface of granules undoubtedly plays a much larger part in solution of casein in alkalies than it does in solution in acids, but penetration of alkaline solutions into the granules and consequent swelling certainly are not entirely absent.

The velocity of solution in aqueous alkalies is directly proportional to the concentration of alkali; it is about the same in sodium, potassium, and ammonium hydroxides but is much slower in solutions of the alkaline earth hydroxides, decreasing in the order strontium, calcium, barium hydroxide.[173] Between 18 and 26°C. the velocity of solution is independent of temperature; at higher temperatures solution is faster in hydroxides of the alkalies and slower in hydroxides of the alkaline earths.[173]

The extent to which casein is dissolved in alkaline solutions is governed chiefly by the proportion of alkali present. If sufficient alkali is present to convert all the casein to alkaline caseinate, casein may be considered indefinitely soluble or miscible with water in all proportions. The maximum concentration attainable in practice is fixed, not by a limit of solubility, but by the mechanical difficulties of dealing with

very viscous solutions and in some cases by formation of jellies. If water is allowed to evaporate from concentrated solutions precipitation does not occur, but instead there is continuous transition through increasingly viscous solution to jelly, hardening and shrinking of the jelly, and finally a horny mass of dry casein.

If sufficient alkali is added to convert all the casein to caseinate, only part of the casein is dissolved. For these reasons the solubility of casein in aqueous alkalies is conveniently considered from the point of view of the proportion of alkali required to make solutions of given concentration and the range of pH attainable in the resulting solutions. The percentage of alkali required is commonly indicated in terms of a "combining equivalent" of casein. It should be remembered, however, that the term cannot be applied to casein in the precise sense in which it is used in physical chemistry and numerically it may differ for different alkalies, different caseins, and different conditions of experimentation.

Aqueous sodium hydroxide in dissolving casein at 25°C. combines with it in the proportion of one gram equivalent for each 2,100 grams of casein.[38] The combining equivalent remains 2,100 through the range in temperature from 21 to 37°C., but from 37 to 60° it changes to 3,700.[159] The solubility is directly proportional to the concentration of the sodium hydroxide regardless of the excess of casein left undissolved in contact with the solution, from which it may be inferred that nearly all the sodium caseinate formed goes into solution and very little sodium hydroxide is withdrawn by the undissolved excess. At 5°C.,[161] however, the undissolved excess does absorb alkali from solution, thereby diminishing the amount available for carrying casein into solution. Under these conditions the solubility is no longer directly proportional to the initial concentration of sodium hydroxide but falls short by an amount that increases with the undissolved excess. In solutions of calcium hydroxide at 25°C. the behavior is like that in sodium hydroxide at 5°. The amount of casein that goes into solution in calcium hydroxide is always directly proportional to the concentration of the alkali remaining in solution, however, and amounts to 1,440[161] to 1,750[171] grams of casein per gram equivalent of calcium hydroxide.

According to Cohn[38] the solubility relations of dry casein dissolving in sodium hydroxide solution may be formulated as though casein were a divalent acid[67, 163] of equivalent weight 2,100, which might be written $R\begin{matrix} \diagup COOH \\ \diagdown COOH \end{matrix}$, forming a highly ionized and highly soluble disodium salt, $R\begin{matrix} \diagup COO^- + Na^+ \\ \diagdown COO^- + Na^+ \end{matrix}$, with a solubility product constant

$\left[R\begin{matrix}COOH\\COOH\end{matrix}\right] \times k_1 \times k_2 = 2.2 \times 10^{-12}$ gram per liter.[38] The symbol in brackets is the concentration of ionized casein in solution and k_1 and k_2 the dissociation constants of the carboxyl hydrogen atoms. Of the 0.11 gram of casein dissolved in water without the aid of alkali 0.097 gram is un-ionized casein and 0.013 gram is ionized casein. The solubility in sodium hydroxide, S, is then

$$S = \left[R\begin{matrix}COOH\\COOH\end{matrix}\right] + \frac{1}{[H^+]^2}\left[R\begin{matrix}COOH\\COOH\end{matrix}\right] \times k_1 \times k_2.$$

The product $k_1 \times k_2$ is 24×10^{-12}, so that casein is a stronger acid than carbonic acid, for which $k_1 \times k_2$ is 18×10^{-18}. It has long been observed that casein displaces carbon dioxide from carbonates.[142, 150]

Cohn's data are reported in some detail because they make it possible for the writer to chart them in Fig. 3 for the purpose of showing roughly the region of pH and concentration of casein that may be realized in practice by dissolving dry casein in alkaline solutions of sodium hydroxide. The data are greatly extrapolated. On adding dry casein to aqueous sodium hydroxide casein passes into solution, consuming alkali, until the concentration of casein and pH reach a point on the curve. Further addition of casein remains undissolved. If more alkali is added the concentration of casein in solution mounts along the curve until all of it is in solution, after which additional alkali increases the pH rapidly. Any desired point in the region below and to the right of the curve can be reached by suitable adjustment of the proportions of the ingredients. Addition of an acid to a solution of casein in alkali decreases the pH along the ordinate of casein concentration, but casein does not begin to precipitate when the curve is reached; all the casein remains in solution until there is less than one gram equivalent of alkali to 8,800 grams of casein.[172, 211]

The solubility of casein in aqueous calcium hydroxide is not governed by the same laws that prevail in sodium hydroxide solutions because only 1,440 or 1,750 grams instead of 2,100 grams of casein are dissolved for each gram equivalent of calcium hydroxide. From the titration curves discussed in the next section it seems that the pH increases less rapidly when casein dissolves in calcium hydroxide than it does during solution in sodium hydroxide. Addition of acids to solutions of casein in calcium hydroxide promptly precipitates casein because the calcium salts formed by reaction are powerful precipitants for casein (see under *Colloidal Behavior*).

The solubility of casein in aqueous alkalies, unlike the solubility in aqueous acids, does not attain a maximum and then fall off again as the pH is increased. Moreover in alkaline solutions the solubility is

increased rather than decreased by the addition of alkali salts in moderate concentration.[223]

Paracasein closely resembles casein in its solubility relations except that it requires somewhat more alkali to effect solution. At 25°C. 1,450 grams

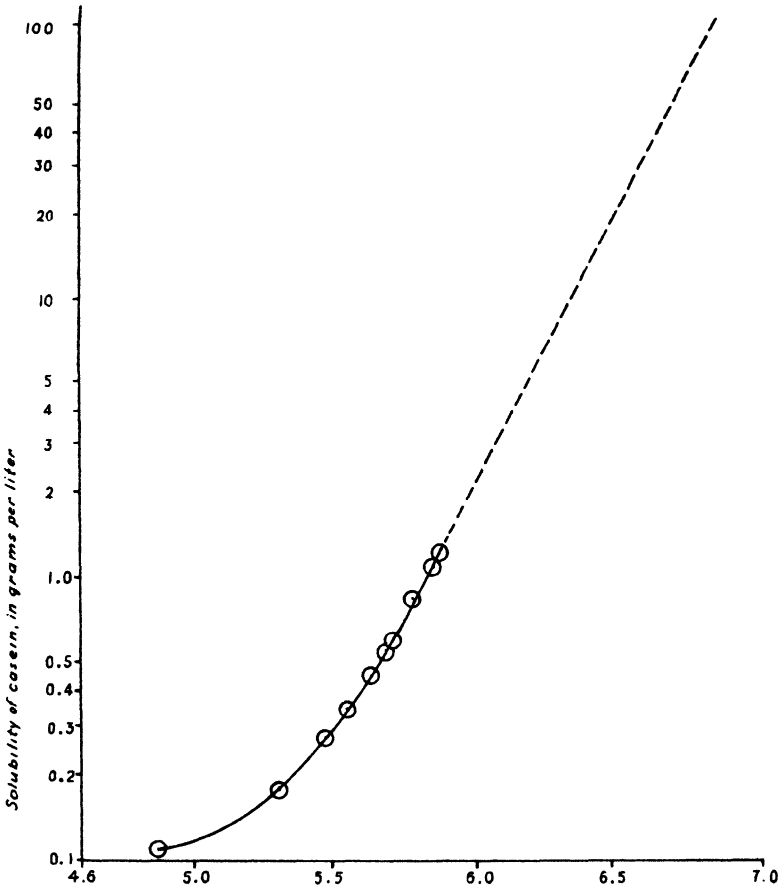


Figure 3.—The solubility of casein in aqueous sodium hydroxide at 25°C. according to the equation of Cohn,[38] which treats casein as a divalent acid with a solubility product constant of 2.2×10^{-13} gram per liter. The encircled points represent experimental data.

of paracasein dissolve per gram equivalent of sodium hydroxide as compared with 2,100 grams of casein. At 5°C. 810 grams of paracasein or 1,300 grams of casein are dissolved per gram equivalent of sodium hydroxide.[160]

There is a slight contraction in volume when casein dissolves in sodium hydroxide. A sample of dry casein whose specific gravity in benzene at 20°C. compared with water at 4° was 1.318 had a specific gravity of 1.42 in solution as sodium caseinate, corresponding to a specific gravity of 1.39 for the dissolved casein.[33]

General Theory of Solubility

Although the proteins are all derived principally from a limited number of amino acids, they exhibit enormous variations in solubility that so far have failed to reveal any systematic relations to molecular complexity such as exists among the fatty acids and other series of organic compounds. Instead there is a complicated behavior much like that of cellulose. Sørensen[195, 196] therefore suggested that, although the peptide linking postulated by Fischer is responsible for the earlier steps in protein synthesis, the proteins themselves should be regarded as mixtures of components held together by secondary valences and capable of reversible association and dissociation.

The more soluble proteins are those that are more highly dissociated electrolytically in solution.[34] When in solution the proteins apparently are hydrated in the sense that a substantial amount of water is bound by the protein and may separate with it when the protein is precipitated, salted out, or crystallized.[194] Pauli and Hofmann[156] and Kondo and Tomiyama,[113] therefore, attributed the solubility of a protein to a balance between hydration, which favors solution, and association, which opposes it. Hydration occurs only at free ionic groups in the molecule. In the peptide linkages the amino and carboxyl groups are bound and capable of little hydration. The diamino acids, however, supply free amino groups and the dicarboxylic acids free carboxyl groups theoretically capable of ionization and hydration.

Casein contains 2.3 times as many carboxyl groups as amino groups of this kind. It is assumed that the free amino groups are saturated by an equivalent number of free carboxyl groups within the molecule or micelle, and that the remaining free carboxyl groups are almost entirely un-ionized as is the case in the higher fatty acids. Under these conditions association dominates over hydration in isoelectric casein and the solubility is slight.

To bring casein into solution effectively hydration must be increased by reagents that increase the number of highly ionized groups. When sodium caseinate, for example, is formed, the un-ionized —COOH groups yield highly ionized $—COO^- + Na^+$ groups, carboxyl groups previously saturated by amino groups within the micelle are opened up, and further ionic $—O^- + Na^+$ groups may be formed at phenolic hydroxyl groups. Solubility is therefore increased enormously by reaction with alkalies. Reaction with acids likewise increases the number of ionic

groups and the solubility but is much less effective because the free carboxyl groups remain largely un-ionized. It may be that the solubility of dried casein is less than that of freshly precipitated casein because the extent of internal neutralization of carboxyl and amino groups increases as the micelles are brought closer together during drying

Titration Curves and Formation of Salts

The binding of alkali by casein does not cease when enough alkali has been bound to carry the casein into solution but continues after solution has been effected until the amount bound is several times that necessary merely to dissolve the casein. The trend in pH of the solution as alkali is added is indicated by the titration curves of Hoffman and Gortner[88] reproduced in Fig. 4, which were obtained by suspending 2 grams of casein in 200 cubic centimeters of water and titrating electrometrically with $1N$ sodium hydroxide or $1N$ calcium hydroxide (using the readily hydrolyzed calcium sucrate to make the equivalent of $1N$ calcium hydroxide), and then titrating back in similar manner with $1N$ hydrochloric acid. The temperature was 22°C.

On titrating with sodium hydroxide the pH increases rapidly from the isoelectric point to pH 6, after which there is a steady binding of sodium hydroxide until a little past pH 7, where there is an inflection in the titration curve marking a region in which the pH again increases rapidly. Near pH 10 the curve once more bends upward as alkali is bound in greater proportion at higher pH. On titrating back with hydrochloric acid the curve is retraced as far as pH 10.5 but then breaks off as the pH falls more rapidly than alkali is neutralized. Neutral salts, such as the sodium chloride formed during the back titration, shift the pH of sodium caseinate solutions in the acid direction, accounting for the hysteresis loop formed by the two curves.[180] It has already been pointed out that neutral salts increase the solubility of casein in sodium hydroxide and shift the isoelectric point in the acid direction.

The titration curve with calcium hydroxide differs somewhat from the curve with sodium hydroxide between the isoelectric point and a point a little past pH 7, but is nearly identical with it in more alkaline regions. Between pH 5 and 6.5 calcium hydroxide is bound in much larger amount than sodium hydroxide. It will be recalled that more calcium hydroxide than sodium hydroxide is required to bring casein into solution.

At 35°C. casein binds less sodium hydroxide than at 22° and at 15° it binds more.[87] Hoffman and Gortner considered the effect of temperature most marked in the region of higher alkalinity, but a recalculation of their data indicates that it may be confined to the region of pH below 8;[168] the calculations involve as yet unsettled assumptions about the effect of protein ions on the activity of hydroxyl ions.

The methods commonly used to calculate the amount of acid or base bound by a protein involve the assumption that the activity coefficient of the acid or base in the solution of protein salt is the same as it is

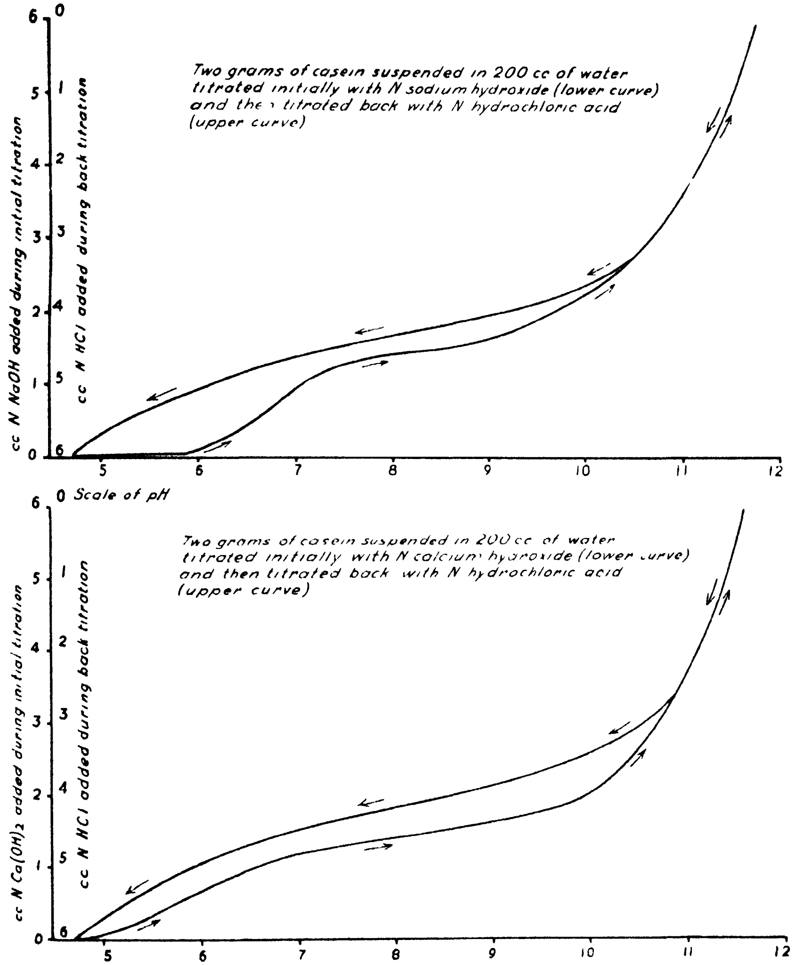


Figure 4.—Titration curves for casein dissolving in sodium hydroxide and in calcium hydroxide (calcium sucrate) and the curves for back titration of the solutions with hydrochloric acid (data of Hoffman and Gortner[88]).

in a protein-free solution of the same concentration. In general, however, the activity coefficient of an electrolyte, γ, is not independent of other electrolytes present but varies with the ionic strength of the solution, $\mu = \frac{1}{2}\Sigma(c_1 z_1^2 + c_2 z_2^2 + \ldots)$ in which $c_1, c_2 \ldots$ are the molar

concentrations of each ion present and z_1, z_2 ... the respective valences.[237, p. 774] The difficulty in applying these considerations to solutions containing proteins is ignorance of the molecular weight and valence of the protein ions. Stone and Failey[236] found that isoelectric proteins have little effect on the activity coefficient of electrolytes but ionized proteins have a marked effect and that, for egg albumin and edestin, the arbitrary assumption of a molecular weight of 11,400 brought reasonable agreement with the formula $-\log \gamma = 0.5 \sqrt{\mu}$, which applies to ordinary electrolytes.[237, p. 791] The molecular weight assumed, however, is only a third of the micellar weight of egg albumin and $\frac{1}{18}$th that of edestin. If Stone and Failey's procedure is correct and is applicable to casein as well as to edestin, the alkali bound by casein agrees closely with that indicated by the titration curves of Fig. 4 up to approximately pH 10.5; but beyond that the true titration curve, instead of bending sharply upward, bends down until beyond pH 12 no further alkali is bound.[163]

Notwithstanding a contrary impression that long prevailed[13, 14, 211] paracasein has nearly the same capacity for binding bases as has casein.[180]

The titration curve for casein in hydrochloric acid is much simpler than the titration curve in sodium hydroxide solution. The curve in hydrochloric acid bends smoothly without inflection, the pH decreasing ever less rapidly as more acid is added.[87]

Formation of Salts of Casein

Earlier investigators who estimated the molecular weight of casein to be roughly 8,800 regarded it as a polybasic acid capable of stepwise replacement of its acid hydrogen atoms with a metal to form series of salts of increasing basicity, just as the tribasic phosphoric acid forms uni-, di-, and trisodium phosphates. Many investigators have reported methods of preparing series of caseinates [208, ch. 3, 4] but the identity of the salts rests on little evidence other than the fact that the product of an arbitrary method of procedure has a composition very roughly approximating the anticipated stoichiometric relation. Caseinates corresponding roughly to the combining equivalents 11, 22, 45, 90, and 180×10^{-5} gram equivalents of base per gram of casein were formerly recognized for the alkali and alkaline earth metals,[14, 120, 179, 211, 212] magnesium,[215] and ammonium radical.[211] The evidence for these combining equivalents consisted chiefly in measurements of the minimum quantity of alkali required to dissolve casein, to neutralize the solution to various indicators, and to hold it in solution on titrating back with acid, together with observations of the electric conductance of solutions containing varying proportions of alkali. Essentially such methods can be traced back to inflections in the titration curves of Fig. 4.

Because of the limited and variable solubility of dry casein in acids and the simpler form of the titration curve most of the earlier investigators concluded that no definite compounds were formed with acids.[213] Robertson,[172] however, believed that a compound is formed with 32×10^{-5} gram equivalents per gram of casein, the quantity of hydrochloric acid required to redissolve immediately casein freshly precipitated from alkaline solution. Hitchcock[85] from potentiometric measurements of the activities of hydrogen and chloride ions concluded that 80×10^{-5} gram equivalents of hydrochloric acid combine with one gram of casein.

Caseinates of the heavy metals such as silver, copper, and lead have been described[122, 147, 157] but they are even less definite than the alkali caseinates. Compounds with organic bases such as pyridine, nicotine, methylamine, arginine, cocaine, strychnine,[127] with aniline dyes,[169, 170] with starch,[10] and with tannin[200] are still more ambiguous.

As a polybasic acid of molecular weight 8,800 casein with 16 free carboxyl groups having dissociation constants at four or five different levels[226] might have satisfied the requirements of the series of combining equivalents postulated if there had not been so much obvious straining of the experimental data to make the combining equivalents bear stoichiometric relations to one another. As estimates of the molecular weight increased, however, the picture became more complicated. A molecule of weight 192,000 would have more than 300 free carboxyl or other acid groups, and to account for the titration curve their dissociation constants must be such that they ionize progressively as the pH increases. Such a picture renders the existence of a series of half a dozen separately identifiable caseinates of sodium, for example, highly improbable. The term sodium caseinate as now used implies no fixed ratio of sodium to casein.

With micelles of the size now known to exist in solutions of casein, purely physical adsorption of acids and bases at the interface between the micelles and the aqueous medium is possible and has been advocated as the mechanism of binding rather than a stoichiometric reaction.[6 (p. 219), 12, 212, 213] The shape of the titration curve in acids and that part of the titration curve in alkalies beyond pH 10.5 is such as would be expected in binding by adsorption. Since free carboxyl groups are undoubtedly present, however, stoichiometric reaction with bases, at least, should certainly be expected, though adsorption might well take place in addition. Hoffman and Gortner,[87, 180] consider that binding of acids and bases is predominantly stoichiometric between pH 2.5 and pH 10.5, but adsorptive below pH 2.5 and above pH 10.5.

Decision between the theories of adsorption and of chemical reaction has been sought in phase rule studies of systems from which water, with its complications of electrolytic dissociation, was excluded. Bancroft and Barnett[7] found that casein forms a true compound with gaseous

hydrogen chloride containing about 0.075 gram of hydrogen chloride per gram of casein, after which more hydrogen chloride is taken up by adsorption; with ammonia, on the other hand, they found only adsorption. Czarnetsky and Schmidt[41] concluded from similar studies that casein forms stoichiometric compounds with both hydrogen chloride and ammonia. In anhydrous ethyl alcohol casein forms a compound with hydrochloric acid and with sodium hydroxide it combines in part stoichiometrically and in part by adsorption.[9]

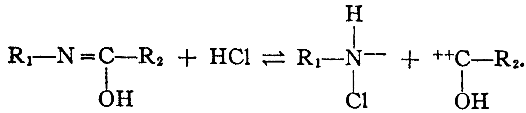
Careful count of the amino acids and phosphorus in casein reveals the possibility that there are from 285 to 420 free acidic groups capable of neutralizing bases if the molecular weight is 192,000.[36, 39] These would suffice to combine with 219×10^{-5} gram equivalents of alkali per gram of casein. Many investigators[36, 72, 179] consider that the maximum binding capacity for alkali is about 180×10^{-5}. The titration curves, however, fail to reveal a maximum binding capacity; instead the amount of alkali bound increases indefinitely as the pH increases until there is more than one equivalent bound for each atom of nitrogen in casein.[87] In solution at pH above 10.5, however, casein begins to hydrolyze, its combining capacity for base is permanently increased; and as already pointed out the customary calculation of bound alkali is erroneous by reason of the effect of the protein ions on the activity coefficient of the alkali. Cohn and Berggren[36] believe that one-third of the available free acidic groups are internally bound by amino groups in the native protein and become released when the protein is dissolved and "saturated" with alkali. Ettisch and Schulz[49] find that only part of the binding of alkali takes place as an instantaneous ionic reaction; the rest occurs as a slow reaction requiring many hours, during which diffusible fragments of the molecule are split off irreversibly. The splitting of the molecule by alkalies is inhibited by neutral salts in the range of pH 7.4 to 11.[49, 180]

Electrolytic Properties of Aqueous Solutions

Aqueous solutions of casein conduct electric current like solutions of strong electrolytes; there is transport of casein through the solutions, and free casein is deposited at the cathode from solutions in acids and at the anode from solutions in bases. At a time when the free carboxyl and free amino groups in casein were deemed insufficient to account for its combining capacities Robertson[179, ch. 8] postulated combination at peptide linkages, splitting the casein into positive and negative ions of equal size according to the scheme:

$$R_1{-}N{=}\underset{\displaystyle OH}{\underset{|}{C}}{-}R_2 + NaOH \rightleftharpoons R_1{-}\underset{\displaystyle OH}{\underset{|}{\overset{\displaystyle H}{\overset{|}{N}}}}{-} + {}^{++}\underset{\displaystyle ONa}{\underset{|}{C}}{-}R_2$$

for the formation and ionization of sodium caseinate and for the formation and ionization of casein chloride:



According to this scheme there are practically no inorganic ions in solutions of casein salts and current is carried in both directions by organic ions. Deposition takes place at one electrode only during electrolysis, because at the other electrode the deposit is redissolved as fast as it is formed by the acid or base likewise set free at that electrode.

It was shown subsequently that the micelles of casein dissolved in acids are all positively charged with transport to the cathode; in bases negatively charged with transport to the anode; and that in an isoelectric zone between pH 3.65 and 4.75 there is very little movement of the casein.[133, 166] Proteins in general combine with cations only on the acid side of the isoelectric point and with anions only on the basic side.[128 (ch. 2), 129] Potentiometric measurements prove that casein solutions contain free inorganic ions; in solution in hydrochloric acid all the chloride ions remain free[84, 85] and in solution in sodium hydroxide the sodium ions remain free.[108] The situation is somewhat more complicated in solutions in alkaline earth hydroxides because part of the inorganic cation remains free while the other part is bound in a complex ion with the casein.[108] The ionization of the salts of casein, therefore, produces either positive or negative ions of casein, and the ions of opposite charge are the simple inorganic ions of the acid or base with which the casein has combined.

During electrolysis solutions of casein obey Faraday's law in that the amount of casein deposited at one of the electrodes is proportional to the amount of electricity passed[69, 73, 174, 176, 179, ch.8] and inversely proportional to the amount of acid or base combined with the casein.[69, 73] The electrochemical equivalent is independent of temperature.[68] The combining equivalent of casein for univalent alkalies calculated from measurements of electric transport is of the order of 2,000 to 2,400 grams per gram equivalent of alkali.

The very large size of the casein ions in comparison with the inorganic ions of opposite charge does not keep the casein ions from carrying a large portion of the current during electrolysis. Measurements of the transport number of the casein anion (the fraction of the total current carried by the casein anions) made by the Hittorf method are recorded in Table 5, together with the mobilities (conductivities at infinite dilution) of the casein anion, the cations, and the salts (anion

TABLE 5.—Transport Numbers of Casein Anion and Mobilities of Ions in Alkaline Solutions of Casein and Derivatives of Casein at 30°C.

Salt	g equiv. alkali per g casein (× 10⁵)	pH of the solution	Transport number of casein anion	—Mobility in mhos of— casein anion	inorganic cation	salt	Reference No.
Of casein:							
Sodium caseinate	56	6 6	0 453	46 2	56 2	102 4	73
” ”	65 2		0 499				144
” ”	70	7 0	0 455				73
” ”	82 5	7 6	0 430				73
” ”	90 5		0 454				144
Potassium ”	62 5	6 9	0 363	46.5	81 4	127 9	73
Rubidium ”	56	6 5	0 355	45 5	84 5	130 0	73
Cesium ”	55	6 5	0 334	43 0	85 3	128 3	73
Magnesium ”	67 5	7 0	0 840				75
Calcium ”	66	7 2	1 00	19 0	66 1	85 2	75
” ”	77	7 7	1 05				75
” ”	95	9 4	0 76				75
” ”	110	10 3	0 64				75
Strontium ”	62 5	6 8	0 73				75
Barium ”	74	7.3	1 24				75
Of racemic casein							
Sodium caseinate	..		0 390	37 0	56 2	93 2	74
Potassium ”	..		0 318	37 7	81 4	119 1	74
Of dephosphorized casein:							
Sodium caseinate	65 0		0 428	37 2	55 8	93 0	144
Calcium ”	74 9		0 542	26 4	66 1	92.5	144

plus cation). In solution in the univalent alkalies the casein ions carry from a third to nearly half of the current and have a mobility comparable with that of small organic ions such as the acetate ion, and greater than that of the ions of amino acids.[144] The mobility increases with the amount of alkali combined with the casein; theoretically the mobility should be directly proportional to the number of hydroxyl ions bound in solutions of the same ionic strength.[2] The mobility of casein anions increases nearly linearly with temperature by about 3 mhos per degree, which is the highest temperature coefficient for any ion recorded in the literature, that for sodium ion being 1.05 and for potassium ion 1.32, for example.[68] The alkali caseinates remain highly ionized even at high concentrations; solutions as concentrated as 8.65 per cent may be considered completely ionized electrolytes[68]

The transport numbers for casein anions in caseinates of the alkaline earths are impossibly high, sometimes exceeding unity. The facts may be explained by assuming that the divalent alkaline earths form complex ions with casein. About half of the calcium, for example, is held un-ionized in the casein anion and travels to the anode while the other half exists as free calcium ion traveling in the opposite direction. In

the calcium salt of dephosphorized casein about 40 per cent of the calcium is retained in the complex casein anion so that the phosphoric acid in casein accounts for only a portion of its ability to form complex ions with the alkaline earths.[69, 75]

Data for the conductivities of solutions of alkali and alkaline-earth salts of casein are reported by several authors with reasonable agreement but with varying interpretations.[61, 74, 120, 152, 153, 154, 179, ch 10] Data of Miyomoto[144] are reported in Table 6. The solutions obey the Ostwald

TABLE 6—Electric Conductivities of Solutions of Casein in Alkalies at 25°C.[144]

	Equivalent conductivity in mhos of caseinate of				
g. equiv. alkali per liter	Sodium	Potassium	Calcium	Barium	Strontium
0.032	...	70.7	9.3	10.2	.
0.024	50.0	74.8	10.6	. .	18.8
0.016	55.0	78.6	12.0	12.8	20.1
0.012	57.7		13.5	.	21.2
0.008	62.0	84.5	16.3	17.0	24.1
0.004	68.5	90.8	22.5	22.7	28.8

50×10^{-5} = g. equiv. univalent alkali per g. casein
80×10^{-5} = g. equiv. divalent alkaline earth per g. casein

dilution law.[152, 153, 154, 179 ch 10] It has also been found empirically that $(C\Lambda)^{1/2} = k\,(1/\Lambda)$ in which C is the equivalent concentration and Λ the equivalent conductivity.[61, 144] The conductivity, in common with that of other colloidal electrolytes, is greater for high-frequency than for low-frequency electric current, the increase being twice as great for alkaline-earth caseinates as for alkali caseinates, from which it has been concluded that the alkaline-earth caseinates are highly dissociated but form associated ions that are conducting for high-frequency but not for low-frequency current.[235]

The extreme view has been expressed that casein ions take no part in the conductivity of casein solutions in alkalies, but that the conductivity is due entirely to the inorganic ions and to decomposition products of casein that can be removed by dialysis.[92, 139]

Optical Properties of Casein Solutions

Specific Rotation

Solutions of casein are optically active, rotating the plane of polarization to the left.[92, 137, 179, ch. 14] For solutions in acids α_D (light of the *D* line of sodium vapor) is reported as $-86°$[168] to $-89°$[224] and α_{5461} (wave-length 5461 Å. of mercury vapor) as $-133°$.[89] For solutions in neutral salts α_D is $-81.5°$ to $-84°$ while for solutions in alkalies α_D varies according to the concentration of the alkali from -86.6 to $-117°$[224] and α_{5461} is reported as high as $-127°$.[89] Complete

removal of fat from casein results in higher specific rotation of its solutions.[82]

With increasing pH the specific rotation increases to a maximum and then falls off slightly as shown in Table 7. On heating the solutions the

TABLE 7.—Specific Rotation of Casein in Alkaline Solutions of Varying pH

Data of Almquist and Greenberg[3]		Data of Pauli and Hofmann[156]	
pH	$-\alpha_D^{25°}$	pH	$-\alpha_D^{25°}$
		5.65	91°
		6.29	97
7.3	97.5°	7.01	102
		7.86	106
8.8	109.8	8.46	108
9.5	114	9.25	110
10.5	115	10.46	115
11.5	121	10.82	120
11.8	125	11.75	125
12.3	122.5	12.14	124
12.6	122	12.85	119
		13.61	114

rotation increases slightly below pH 11.7 and decreases above pH 11.7.[156] In solutions in univalent alkalies the maximum apparently is a trifle higher and at slightly higher pH than in solutions of alkaline-earth hydroxides.[224] It is reported that α_{5461} is −127° both at pH 6.0 and pH 8.0.[89]

At a given pH the specific rotation increases as the concentration of casein in solution decreases.[28, 224] According to Carpenter[28] α_D for monodispersed fraction of casein of micellar weight 96,000 at pH 6.8 is −99° when the concentration of casein is 1.5 per cent, but on dilution to less than 0.7 per cent it increases to −105° at 0.15 per cent. Ultracentrifugal analysis indicated that the micelles dissociate on dilution below 0.7 to micelles of one-third the initial size, namely 32,000. The dissociation is completely reversible because the micelles go back to 96,000 on restoring the original concentration. Carpenter's findings apparently confirm Sørensen's[193, 194] conception of casein as a complex undergoing reversible association and dissociation. The specific rotation of the cleavage products obtained on hydrolysis of casein is about half that of casein.[40, 99]

The specific rotation of solutions of commercial casein in 10 per cent sodium acetate solution varies considerably.[65] Samples of hydrochloric acid casein varied from α_D −72.6° to −92.2°, lactic acid casein from −74.4° to −89.9°, and sulfuric acid casein from −61.7° to −72.2°. An excess of acid during precipitation of the casein tends to reduce the specific rotation, but the temperature at which the casein is dried has little effect.

Index of Refraction

Within certain limits the index of refraction of aqueous solutions of casein may be expressed by the equation $n - n_1 = 0.00152c$, in which n is the index of refraction of the solution, n_1 that of the solvent, and c the percentage of casein in solution.[172, 175, 185] The equation applies independently of the nature of the acid or alkali and independently of temperature between 20° and 40°C., but for nonaqueous solvents the constant term changes with the solvent. Extrapolation of the equation indicates that the refractive index of pure casein should be 1.675.

The equation is based on the procedure of dissolving increasing quantities of casein in a solution of given strength, say 0.1*N* sodium hydroxide, in which case the pH varies systematically with the concentration of casein. When the pH is altered in solutions of fixed concentration of casein it is found that the refractive index varies in a manner parallel with the change in viscosity.[111, 114] Another relation apparently applicable to solutions of casein is Wiegner's equation $n = c_1 \ [(n_2 - n_1)/d_2] + n_1$, in which n, n_1, and n_2, are the indexes of refraction for the solution, the solvent, and the dispersed micelles of casein respectively, d_2 is the diameter of the dispersed micelles, and c_1 is a function of the concentration of casein.[47] In dilute solutions d_2 decreases with further dilution. In 0.1 and 0.2 per cent solutions d_2 likewise decreases when the solution is frozen, but in one per cent solutions freezing increases d_2.[239]

Double Refraction during Flow

Solutions of casein, while flowing, become optically anisotropic and exhibit double refraction,[240] which arises from orientation of rod-shaped micelles with their long axes parallel to the direction of flow. The optical behavior of solutions of sodium caseinate containing 40 or 50 grams of casein per liter is that of a polydisperse system of micelles whose length is somewhat greater than their width. Addition of neutral salts to the solutions may bring about changes in both size and shape of the dispersed particles by causing the micelles to group together in bundles with their long axes parallel or by causing such bundles to break up into the separate micelles. Thus additions of sodium chloride or of sodium sulfate increase the double refraction during flow, but addition of potassium iodide decreases it.

Scattering of Light

The micelles in solutions of casein in both acids and alkalies are large enough to scatter light and therefore to reveal their presence in an ultramicroscope. The micelles seem to be larger in acid than in basic solutions and those of casein larger than those of paracasein.[13] Even solutions that appear transparent as ordinarily observed exhibit

a distinct Tyndall cone when they are properly illuminated. The scattered light complies with Rayleigh's law, in which $I\ \lambda^4/I_0$ is constant, I_0 and I being respectively the intensities of incident and of scattered light of wave-length λ.[91] The scattered light is depolarized.[115, 164, 202] Addition of a salt such as sodium chloride increases I by dehydrating the micelles, increasing their refractive index. The micelles of calcium caseinate appear to be less hydrated than those of sodium caseinate. On heating solutions of calcium caseinate the micelles are further dehydrated and the solutions become turbid but are restored to the initial condition on cooling again. Solutions of sodium caseinate between pH 9 and pH 13 are not similarly altered by heating but become so at lower or higher pH.[91, 156] A method of qualitative analysis of proteins including casein has been based on measurement of the turbidity produced by increasing concentrations of ammonium sulfate.[186]

Absorption of Ultraviolet Light

Solutions of casein in alkalies absorb light in a band of wave-lengths in the region of the ultraviolet. In 0.02*N* sodium hydroxide the absorption band occurs at λ2975 to 2407 Å.[1, 140] Solutions of paracasein exhibit an absorption band in the same region.[241] The band shifts toward longer wave-lengths in more strongly alkaline solutions and the absorption curve is modified in a manner suggesting a change in the structure of the casein.[42] The action of ultraviolet light changes casein to a substance that is not precipitated by acetic acid.[4]

Viscosity of Casein Solutions

Aqueous solutions of casein are relatively viscous even at comparatively low concentrations. As the concentration of casein increases, the viscosity increases even more rapidly. As the temperature increases,

TABLE 8.—Viscosity of Aqueous Solutions of Sodium Caseinate

(Data of Chick and Martin[33])

As a function of concentration		As a function of temperature for a solution containing 93.9 g. per l.	
Grams casein per liter	Relative viscosity at 25°C. (H_2O = 1)	Temperature, °C.	Relative viscosity
21.7	1.82	0	145
43.5	3.37	9.75	72.5
60.5	6.12	23.0	26.7
70.6	8.48	30.6	17.6
84.9	13.66	40.0	11.4
93.9	23.72	51.7	7.30
		60.2	5.55
		72.7	3.90
		81.25	3.12
		99.6	2.12

the viscosity falls off rapidly. Typical data for sodium caseinate are given in Table 8.

Solutions of casein in acids or alkalies containing a few per cent of casein are truly viscous in the sense that they conform to Poiseuille's law, the rate of flow being directly proportional to the pressure causing it.[91, 116, 118] Sodium caseinate solutions containing 9 per cent casein at pH 9.1 to 9.2 are truly viscous[230] but solutions in alkaline salts and

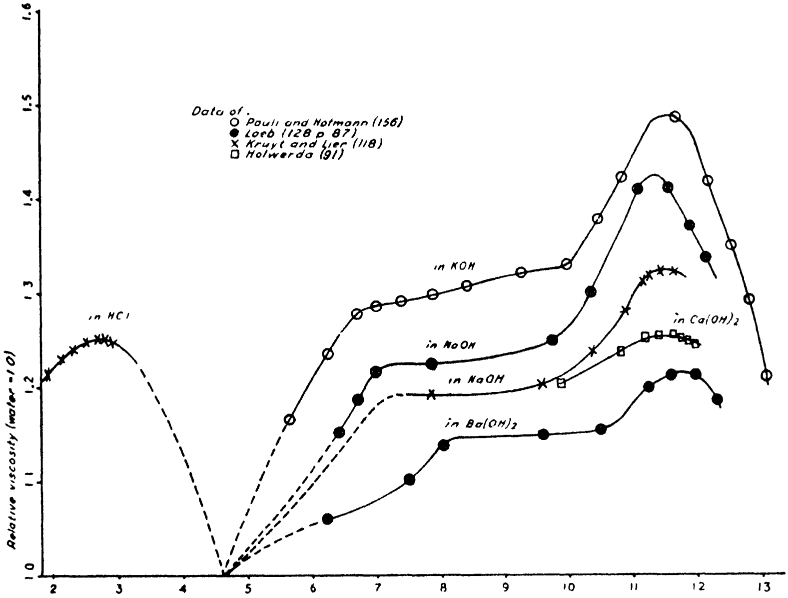


Figure 5.—Variation with pH of the relative viscosity of 1 per cent solutions of casein in hydrochloric acid, potassium hydroxide, sodium hydroxide, barium hydroxide, and calcium hydroxide.[91, 118 (128, p. 87), 156]

univalent alkalies containing 12 per cent or more of casein are pseudoplastic,[18, 21, 218, 219] as are also dilute solutions of casein containing agar.[62] On the other hand solutions of as much as 28 per cent casein containing both sodium and calcium hydroxides and of pH between 12 and 13.5 (typical casein glues) are truly viscous with a viscosity increasing rapidly with time until they begin to form jellies.[18] Apparently solutions of the caseinates of univalent metals change from viscous to pseudoplastic at much lower concentration than solutions of the caseinates of bivalent metals.

The viscosities of one per cent solutions of casein in hydrochloric acid, potassium hydroxide, sodium hydroxide, barium hydroxide, and calcium hydroxide over the range in pH from 2 to 13.6 are shown in Figure 5. Maxima in viscosity occur at pH 2.6 and near pH 11.5

and the curves point toward a minimum at the isoelectric point. These *maxima remain the same over the range in concentration from 0.5 to 1.5 per cent casein.*[91, 118] *At a given pH and concentration of casein the viscosities of the alkaline caseinates decrease in the order potassium, sodium, calcium, barium. Addition of neutral salts decreases the viscosity of casein solutions at constant pH; in acid solutions the effect* of a salt increases markedly with the valence of its anion, whereas in alkaline solutions it is the cation whose valence is effective.[22, 90, 118]

In 9 per cent solutions of casein in alkalies the relation between viscosity and pH as shown in Figure 5 differs markedly from the relation in one per cent solutions. The curves rise rapidly to a maximum at pH 9.2[227] or a little beyond[230] after which, with the exception of ammonium caseinate, they fall off precipitously to a comparatively low viscosity at pH 11.5, where the dilute solutions exhibit their maxima.[227] The solutions in ammonium hydroxide, however, remain at maximum viscosity from pH 9.2 to pH 11.5. Solutions of casein in hydrolyzing salts such as trisodium phosphate, sodium fluoride, sodium silicate, sodium carbonate, and sodium arsenate exhibit sharp maxima at pH 9.2, but in borax the maximum occurs at pH 8.2.[225, 227]

The viscosity of commercial caseins varies widely according to the method of preparation and the ash content of the product. Casein heated above 60°C. during its preparation gives more viscous solutions than unheated casein[227] though heating the alkaline solutions themselves decreases the viscosity slightly.[156] Alkaline solutions of casein on long standing in sterile condition decrease slightly in viscosity without change in pH or in optical rotation.[156]

Surface Tension, Surface Films, and Emulsions

The surface tension of freshly formed surfaces of solutions of casein in acids or alkalies is the same as that of water for about 6 seconds, after which it decreases systematically for many hours.[102] Apparently the casein concentrates in the surface but it attains its equilibrium condition slowly.

Under suitable conditions casein spreads readily in very thin films on water. On water acidulated to pH 1 to 2 the films are "monomolecular" in thickness, 0.6 to 0.75 mμ thick,[63] and cover an area of 1.27 square meters per milligram.[64] Above pH 2 the films are thicker and on distilled water they are three times as thick. On strongly acidulated water the films spread promptly but they spread slowly on distilled water. Spreading takes place more readily at higher temperatures, in contrast to gelatin, which spreads better at lower temperatures. Proteins freely spreading on water assume "expansion patterns" character-

istic of the protein, that of casein being classified as "smooth circular pattern."[242] The casein micelles may be considered as flat platelets or needles oriented with the polar CO—NH groups toward the water; the thickness of the film then depends upon the length of the constituent amino acids.[63]

Because of its property of forming films at the surface of water casein can be used as a stabilizing agent for emulsions. Emulsions are of two types, oil dispersed as droplets in water or water dispersed as droplets in oil. Under appropriate conditions casein stabilizes either type.[238] With crude petroleum oil casein forms emulsions of oil-in-water unless the petroleum contains tar or asphalt, which stabilizes emulsions of water-in-oil.[138] With petroleum distillates the type of emulsion depends upon the specific gravity of the distillate. If the specific gravity is less than 0.820, fine, stable oil-in-water emulsions are formed; between 0.820 and 0.828 coarse, unstable emulsions; between 0.828 and 0.857 no emulsions; 0.857 to 0.860 moderately stable water-in-oil emulsions; and above 0.869 fine, stable water-in-oil emulsions.[188] In the region of instability the type of emulsion may be reversed by altering the relative volumes of oil and water; or the method of preparation and the stability and type of emulsion may be modified by adding electrolytes to the water. The stabilizing films at the interface between oil and water vary greatly in thickness from possibly monomolecular films to optically visible films.[188]

Vegetable and fish oils, such as olive, sperm, castor, poppy seed, and cod liver oils form fine, stable emulsions of water-in-oil stabilized by casein. Linseed oil forms a dual emulsion in which both types are present but the water-in-oil type is the more stable. These emulsions can be reversed to the oil-in-water type by adding sodium hydroxide, and then brought back to the original type by adding barium chloride or barium hydroxide.[189]

Casein is likewise an effective protective colloid for stabilizing dispersions of lyophobic substances. For example, sols of colloidal gold protected by casein require much higher concentrations of electrolytes for coagulation than do the unprotected sols.[228] The casein coats the dispersed particles and imparts to them its own lyophilic characteristics. The action of casein in protecting the dispersion of calcium phosphate in milk has been pointed out in Chapter 1.

Colloidal Behavior

As early as 1843 Selmi[190] recognized that solutions of casein are not ordinary solutions but are made up of small particles dispersed in the liquid. Eighteen years later Graham,[66] laying the foundations

of colloid chemistry, introduced the term "sol" for such solutions; the precipitates or jellies obtained from them he called "gels" and the process of forming sols "peptization." Graham had the proteins particularly in mind when he coined the word "colloid," which means glue-like.

The distinguishing characteristics of colloids have always been associated with the dispersed particles or micelles which are large as compared with the molecules or ions in solutions of ordinary crystalloids, though small as compared with macroscopic particles that can be resolved and observed clearly with a microscope. As a rule micelles are conceived as aggregates of molecules or ions that may vary in size and structure in contrast to the unvarying identity of the molecules or ions of any one substance. Colloidal characteristics, however, are largely a consequence of the size of the dispersed particles; whether the particles are aggregates of molecules or macromolecules (very large molecules) of identical composition is of minor importance.

The scattering of light, ultramicroscopic visibility, and ultracentrifugal sedimentation of dispersed casein, which are consequences of the relatively large size of the micelles, have already been described. Diffusion of casein micelles is much less rapid than that of the ions of crystalloids.[20] Membranes of suitable porosity, such as collodion, prevent the diffusion of casein while permitting dissolved crystalloids to pass freely so that separation of casein from crystalloids can be effected either by ultrafiltration[57, 70] or by the process of dialysis, in which the solution outside the membrane is continuously replaced with pure water to remove the crystalloids as they pass through. If the solution outside the membrane is not replaced an equilibrium is finally reached when the concentration of crystalloid in the outer solution is such that the permeable ions or molecules pass through the membrane in both directions with equal rapidity. At equilibrium, however, the distribution of the ions of inorganic electrolytes on the two sides of the membrane is unequal because some of the ions are bound by the casein in the form of salts. Alkaline sols of casein retain calcium more effectively than sodium because calcium caseinate is much less highly ionized than sodium caseinate.[221] Casein itself can be formed into membranes[23, 136] impermeable to colloids but permeable to crystalloids; membranes of casein permit sodium ions to pass more readily than potassium ions and calcium ions less readily than either.[23]

The Donnan Equilibrium[45]

If solution I of sodium caseinate, NaCas, is separated from solution II of sodium chloride by a membrane impermeable to Cas^- but permeable to Na^+ and Cl^-, both thermodynamic and kinetic reasoning show

that equilibrium is reached when the product of the activities of sodium and chloride ions is the same in **I** and **II**, that is,

$$[Na^+]_I \times [Cl^-]_I = [Na^+]_{II} \times [Cl^-]_{II}.$$

In **I** there are more sodium ions than chloride ions because

$$[Na^+]_I = [Cl^-]_I + [Cas^-]_I$$

while in **II**

$$[Na^+]_{II} = [Cl^-]_{II},$$

therefore

$$[Cl^-]_I([Cl^-]_I + [Cas^-]_I) = [Cl^-]_{II}^2$$

and

$$[Na^+]_I([Na^+]_I - [Cas^-]_I) = [Na^+]_{II}^2.$$

If there is more than one diffusible electrolyte in the system the product of the activities of any pair of anions and cations is the same in **I** and **II**. For uni-divalent electrolytes such as sodium sulfate the product in question is $[Na^+]^2 \times [SO_4^{--}]$. The difference in activity of ions in the two solutions gives rise to a difference in electric potential across the membrane, the membrane potential E, expressible in volts by the equation

$$E = \frac{RT}{F} \log_e \frac{[Cl^-]_{II}}{[Cl^-]_I}$$

in which R is the gas constant, T the absolute temperature, and F the Faraday equivalent.

Proctor and Wilson[167] applied Donnan's principle to the effects of electrolytes on the behavior of protein jellies. The linking together of the micelles in the structure of the elastic jelly prevents their diffusion as effectively as a semipermeable membrane. The jelly and solution contained within it may therefore be regarded as solution **I** and the solution outside the jelly as solution **II**. The excess of diffusible ions in the jelly sets up a difference in osmotic pressure as a result of which water is drawn into the jelly and it swells against the restraint imposed by the elastic cohesion of the jelly structure, which conforms to Hooke's law. Wilson[220] further extended the conception to protein sols by considering the micelles as minute elastic jellies made up of networks of amino acids containing imbibed solution, the micelles being equivalent to solution **I** and the dispersion medium to solution **II**.

Loeb[128, 130, 131, 132, 134] and others[70, 201] applied the principle of the Donnan equilibrium to the behavior of casein with much success, particularly with respect to osmotic pressure, membrane potentials, viscos-

ity, and swelling of jellies. All these properties are minimum at the isoelectric point where there is little or no combination of casein with electrolytes and consequently little difference in concentration of the solutions within and without the micelles. On increasing the pH by adding alkali, sodium caseinate is formed and the activity of ions within the micelles increases more rapidly than that of the dispersion medium; swelling or dispersion, viscosity, and osmotic pressure increase correspondingly. As further addition of alkali reaches the point of saturation of the combination with casein, the relative difference in activity of ions within and without the micelles passes through a maximum and then falls off, and the viscosity and osmotic pressure do likewise. On the acid side of the isoelectric point there are similar trends except that the casein forms cations instead of anions. At any pH, addition of neutral salts depresses swelling, viscosity, and osmotic pressure by decreasing the relative difference in activity of ions inside and outside the micelles.

Hydration, Electric Charge, and Stability

The concept of the micelles as minute osmotic jellies subject to a Donnan equilibrium represents but one of several schools of thought about the colloidal behavior of the proteins. Much of the rivalry between schools reduces essentially to quibbling about terminology[203, p. 11] because the points of difference lie beyond the limits of present tools for experimentation. Instead of considering the micelles as jellies containing much osmotically imbibed water their association with water may be looked upon as a kind of "hydration" of the casein. The water of hydration is regarded variously as bound more or less chemically,[155] dissolved in the casein,[53, 54, 55] or adsorbed in "shells" around the particles of casein.[117, 118, 119] As far as the theory of the colloidal behavior of casein is concerned the essential point is merely that the micelles as hydrodynamic units moving within the dispersion medium carry with them a relatively large proportion of water, often many times the volume of the casein. The hydration is largely responsible for the high stability of sols of casein and for their classification with the emulsoid as distinguished from the suspensoid colloids.

The stability of sols of casein is governed also by the electric charge carried by the micelles. Many investigators consider that the charge is entirely that of the casein ion formed by the dissociation of a casein salt, in which case dispersed casein is a colloidal electrolyte.[51] Others attribute the charge to adsorption of ions at the interface between the micelles and the dispersion medium[6] or to both ionic dissociation and adsorption.[87, 180] In any case there is an electric double layer and an

electrokinetic potential across the interface which, in addition to determining electrolytic mobility or cataphoresis, contributes to the stability of the sols and gives them some of the properties of the suspensoid colloids.

The interrelation of hydration and electric charge in determining the colloidal behavior of sols of casein may be illustrated by Smoluchowski's[192] theoretical equation for viscosity:

$$\frac{\eta}{\eta_0}=1+2.5\phi\left[1+\frac{1}{\kappa\eta_0}\left(\frac{\zeta\epsilon}{4\pi r}\right)^2\right]$$

in which η and η_0 are viscosities of the sol and the dispersion medium, ϕ the fraction of the total volume occupied by the micelles, r the radius of the micelles, ζ the electrokinetic potential, κ and ϵ the specific electric conductivity and the dielectric constant of the medium. The viscosity depends upon the volume fraction ϕ and upon an electroviscous effect governed by the electrokinetic potential and the electrical characteristics of the medium.[117, 119] Both ϕ and ζ are minimum at the isoelectric point of casein and increase at lower and higher pH to maxima beyond which ζ is diminished by the increasing concentration of electrolyte in the dispersion medium. Addition of electrolytes reduces the viscosity by decreasing ζ,[240] the electrolytes being effective in lower concentrations the higher the valence of the ion of charge opposite to that of the micelles. Nonelectrolytes such as alcohols, on the other hand, affect the viscosity by altering the hydration and the volume fraction ϕ.

Presumably changes in the swelling or hydration of the micelles involve corresponding changes in their size. In addition changes in size take place by joining together or breaking up of micelles, but the little that is known as yet about that subject has already been described in the section on fractionation by ultracentrifugal sedimentation. Egg albumin, which has been examined in the ultracentrifuge over a wide range of pH,[191] is dispersed in micelles that retain their identity between pH 3 and 9, but below pH 3 form aggregates of denatured protein and above pH 9 break down by hydrolysis. Casein may behave similarly except that there is a zone of aggregation near the isoelectric point. The maxima of viscosity in both the basic and acid regions occur after these limits of probable stability of the micelles of casein have been passed.

Coagulation

Coagulation of sols of casein takes place when either hydration or electrokinetic potential of the micelles or both are sufficiently reduced. At the isoelectric point both are minimum and coagulation takes place

rapidly. More highly hydrated proteins, such as egg albumin, remain dispersed even at their isoelectric points. On adding acids to alkaline sols of casein coagulation sets in somewhat before the isoelectric point is reached. The width of the zone of coagulation and the character of the curd formed vary greatly with the nature of the acid.[54, 94] When the zone of coagulation is wide a softer, bulkier, and more gelatinous curd is formed than is the case when the zone of coagulation is narrow. Acids that swell and dissolve dry casein most readily coagulate it in a narrow zone and in a more compact curd. Apparently the anion of the acid exerts an effect on the degree of hydration of the micelles; those acids whose anions promote a high degree of hydration maintain stability of the dispersion in a region of pH where less hydration leads to coagulation.

The stabilizing charge of the casein micelles can be reduced and coagulation effected through the addition of electrolytes. The concentration of electrolyte required for coagulation is less, the higher the valence of the ion of charge opposite to that of the micelles. In acid sols containing positively charged micelles electrolytes with di- or multivalent anions are most effective. For example, one per cent sols of casein at pH 2.2 do not coagulate until the concentration of sodium chloride, sodium nitrate, or calcium chloride is made $0.125M$ but coagulate when sodium sulfate is $0.031M$.[134] In sols containing negatively charged micelles electrolytes with di- or multivalent cations are most effective. In one per cent sols of casein at pH 7 coagulation takes place when calcium chloride is $0.030M$, but with sodium chloride, ammonium chloride, or ammonium sulfate it does not occur until the electrolyte is several times molal.[134] The inverse relation between concentration of electrolyte required for coagulation and the valence of the ion of charge opposite to that of the micelles is characteristic of suspensoid colloids, which are stabilized by charge only and are not hydrated. On the other hand the very high tolerance of alkaline casein sols for electrolytes with univalent cations is indicative of the intermediate position of casein between emulsoid and suspensoid colloids.

Addition of alcohols to alkaline sols of casein decreases the hydration of the micelles. As the concentration of alcohol increases the sols become less emulsoid in character, are coagulated by smaller concentrations of electrolytes, and follow the valence rule more exactly in coagulation.[95] At a sufficient concentration of alcohol that varies with the pH of the sol, coagulation takes place even without electrolytes.[98] Above this first zone of coagulation there is a region of higher concentration of both alcohol and electrolyte in which stable sols are again produced that can be coagulated once more at still higher concentrations of alcohol or electrolyte.[96] The second zone of stability following one of coagula-

tion is frequently observed among colloids and usually involves a reversal of charge, in which case the micelles of casein would be positively charged although on the alkaline side of the isoelectric point.

The coagulating concentration of different alcohols for alkaline sols of casein is lower, the smaller the dielectric constant of the alcohol.[101] On the other hand nonelectrolytes that raise the dielectric constant of water, such as aminoacetic acid, urea, acetamide, and formamide, stabilize casein against coagulation by electrolytes.[97]

Casein coagulates upon addition of formaldehyde to its sols. The formaldehyde acts chemically upon the casein as discussed in Chapter 2, the product being insoluble and incapable of dispersion under the conditions prevailing in casein sols.

Salts of di- and multivalent cations can often be added in considerable proportions to strongly alkaline casein sols because the corresponding hydroxides are of limited solubility and precipitate before coagulation takes place. As a rule the metal hydroxides are promptly protected by the casein and remain dispersed in complex micelles with the casein. For example, when copper salts are added to casein glues, green insoluble copper hydroxide can be seen momentarily at the point of contact, but it rapidly disappears and the glue takes on the deep blue color of colloidally dispersed copper hydroxide. The hydroxides of the alkaline-earth metals, however, are sufficiently soluble to leave enough divalent cation in solution for coagulation to take place slowly. In sols of sufficient concentration of casein, slow coagulation under these conditions forms a jelly rather than a gelatinous precipitate.

Casein Gels and Jellies

In rapid coagulation casein separates either in soft, swollen granules or in gelatinous curds. The granules or curds are in reality small elastic jellies containing much water which shrink and harden as the water is removed in drying. Slow coagulation, if enough casein is present, usually turns the entire sol into a jelly. On concentrating casein sols by allowing water to evaporate the sols become increasingly viscous and finally acquire the firmness of a jelly. The general term gel may be used to include both jellies and precipitates that are made up of small particles of jelly. The transition from sol to gel is not marked by any abrupt point of change in physical properties equivalent to a freezing point except possibly a change from truly viscous to pseudoplastic flow.[17] The ready transition from viscous sols to elastic gels without abrupt change in other properties is one of the characteristics determining the usefulness of casein in glues, paints, and paper coatings, while the elastic nature of the gels is fundamental to its use in plastics.

Casein gels may be reversible or irreversible according to the con-

ditions under which they are formed. Reversible gels are peptized *again, passing back to sols, when the coagulating agent is washed out or diluted with water below the concentration necessary for coagulation* and the pH, *if* altered during coagulation, is restored to a region in which sols are stable. Casein coagulated by acids is readily peptized, or redissolved, by alkalies. The gels formed by electrolytes with univalent cations are peptized by washing out the electrolyte or merely by sufficient dilution. Gels formed by alcohols are peptized in the same way. On the other hand the gels formed by formaldehyde or by sufficient quantities of electrolytes with di- or multivalent cations are irreversible and cannot be peptized by washing or dilution.

In certain regions of concentration and pH, freshly formed gels of casein contract and squeeze out some of the liquid phase, a process known as syneresis.[218, 219]

On drying gels shrink materially in volume and become mechanically stronger and harder. When thoroughly dry they often become very brittle. Dry gels are known as xerogels. As a rule xerogels reabsorb water when exposed either to damp air or to water and in so doing swell and gradually weaken. With inelastic gels such as silica gel the regain of moisture, volume, and mechanical properties lags far behind the loss on drying and varies markedly with the extent to which drying took place before reabsorption began.[210, 229] With elastic gels the hysteresis between the curves for drying and regain is much less marked and it is practicable to consider that the gel, regardless of its previous history, comes roughly to a condition of equilibrium of moisture content, volume, and mechanical properties with the activity of water in the environment.[105] Such typically elastic gels as gelatin, however, still reveal clearly measurable hysteresis.[231, 234] Casein gels are commonly considered elastic[16] although the curves for drying and reabsorption do not coincide exactly;[59] but some casein glues that set to interlocking jellies of casein and silica seem to be distinctly inelastic (see Chapter 8).

The relation between the moisture content of casein gels and the relative humidity of the environment with which they are in equilibrium at 25°C. is indicated in Figure 6.[16, 59, 104] The curves are sigmoid in shape, as is the case with gels in general, showing that the xerogel absorbs moisture in considerable quantity even at very low relative humidity, the absorption increases less markedly with increase in humidity through an intermediate range in humidity, and finally absorption increases very markedly as saturation is approached. Near its isoelectric point casein is less hygroscopic (absorbs less moisture at a given relative humidity) than agar but more so than cellulose. The hygroscopicity is minimum at the isoelectric point and increases with alkalinity. Sodium caseinate is more hygroscopic than calcium caseinate at the same pH.

Briggs[16] considers that the hygroscopicity is the sum of that of the isoelectric casein and of the inorganic ions bound to the protein; since sodium caseinate is more highly ionized than calcium caseinate it is more hygroscopic. The hygroscopicity of casein is not greatly affected

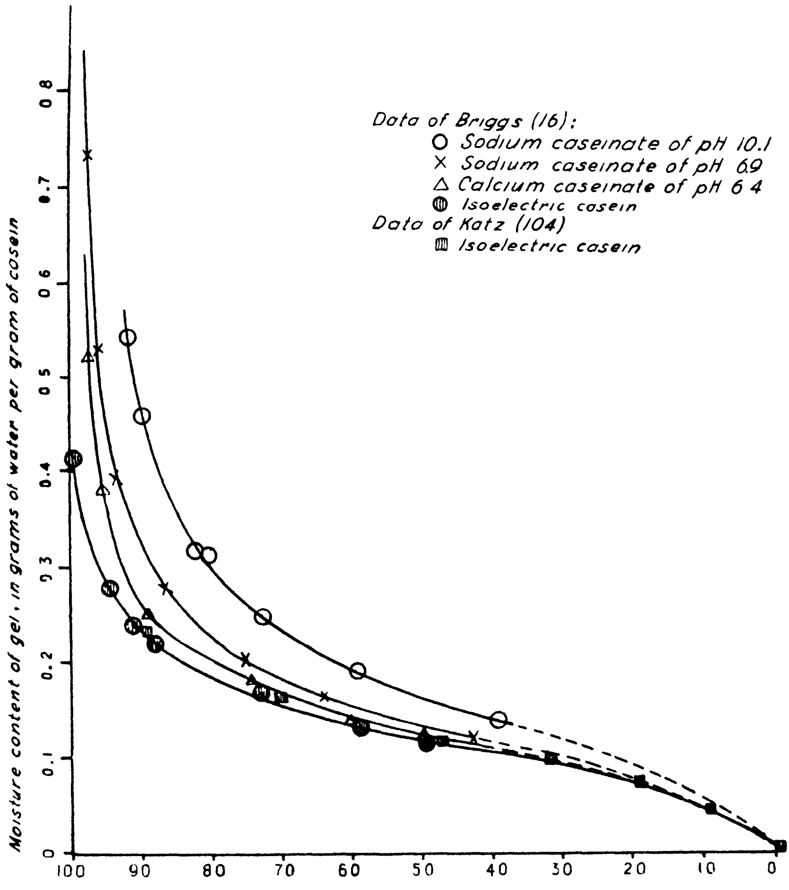


Figure 6.—Relation between relative humidity of the air and the moisture content of gels of isoelectric casein, sodium caseinate, and calcium caseinate at equilibrium at 25°C.[16, 104]

by temperature between 0 and 37°C.,[16] but it is slightly higher, the lower the temperature.[59]

Heat is evolved during the swelling of casein by water,[104] particularly during the absorption of the first portions of water, as indicated by Figure 7. About 25 calories are released in the saturation of one gram

of casein. There is also a slight contraction in the total volume of the system, most of which likewise occurs in the early stage of swelling. The differential heat of swelling, ΔH, which is the heat evolved when

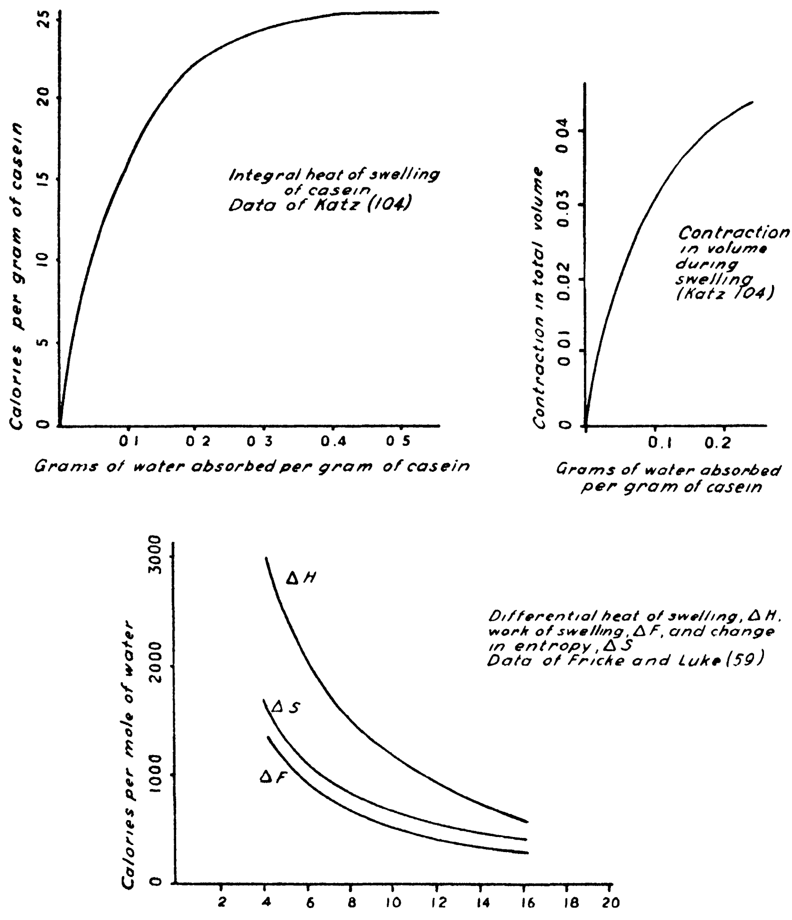


Figure 7.—The integral and differential heats of swelling, work of swelling, change in entropy during swelling, and contraction in total volume during swelling of casein with water.[59, 104]

one mole of water is absorbed by an infinite amount of casein at a given moisture content, varies with moisture content as indicated in Figure 7.[59] ΔH is related to the change in free energy, $-\Delta F$, and the change in entropy of the system, ΔS, by the equation

$$\Delta S = [\Delta H - (-\Delta F)]/T$$

in which T is the absolute temperature. ΔS is of about the same magnitude in the swelling of such diverse colloids as keratin, nuclein, starch, agar, wood, cotton, and even for the solution of water in sulfuric acid.[198] It is probably a property of water, perhaps an orientation or compression of the molecules of water adsorbed on the surface of the swelling material.[106]

The dielectric constant of "dry" casein (probably air dry at approximately 8 per cent moisture content) is 8.0.[60] The specific dielectric polarization, P_ϵ, which is related to the dielectric constant, ϵ, by the formula

$$P_\epsilon = \frac{\epsilon - 1}{\epsilon + 2} \cdot \frac{1}{d}$$

in which d is the density, varies from 0.376 for the xerogel to 0.759 at 20 per cent moisture content, the curve showing an inflection at 6 per cent moisture content.[79] The electric conductivity is low up to 20 per cent moisture content and then varies as follows:[79]

Per cent moisture.........	21.44	22.94	25.08	27.76	30.88	35.64	41.3	49.06
Conductivity, mhos $\times 10^6$.	0.065	0.25	0.75	1.85	7.9	28.6	70.0	166

These facts suggest that the first 6 per cent of water in casein gels is taken up by a mechanism differing from that of succeeding water and that there is a marked change in the structure of the gel above 20 per cent.

Casein gels are plastic when not too dry. The curves of velocity of flow vs. pressure up to 2,000 kg. per sq. cm. are strongly curved parabolas.[80] Below 20 to 25 per cent moisture content flow takes place exceedingly slowly, but above that moisture content the velocity increases markedly, a fact of great importance in the manufacture of casein plastics.

Hydrolysis of Casein

Casein in aqueous solutions and in moist jellies is subject to decomposition by chemical hydrolysis, by enzymatic hydrolysis (biochemical digestion), and by the attack of microörganisms. Dry casein can be kept unchanged for years provided insects and rodents are excluded. Neutral solutions of sodium caseinate remain fairly stable for some months at room temperature if carefully preserved from bacteria and fungi[199] but even then some hydrolysis takes place because dialyzing the solutions slowly removes simpler organic compounds containing nitrogen.[52, 139, 199]

Theoretically the enormous molecules of protein during hydrolysis are first broken at points well within the structure, yielding somewhat smaller but still very large molecules, and these in turn break down in much the same fashion until ultimately the individual amino acids are set free. The picture is one of casein splitting progressively into metacaseins, caseoses,

peptones, peptides, and finally amino acids. The products of the various stages of decomposition prior to the amino acids and some of the peptides are differentiated by arbitrary criteria of solubility or precipitability rather than by criteria of chemical individuality.[83, 92, 179]

Enzymes effect hydrolysis with a certain purposefulness that follows roughly the theoretical picture of step-wise degradation even when the enzymatic hydrolysis is performed *in vitro*. There is no such purposefulness in chemical hydrolysis. Instead there is from the start a "nibbling at the circumference of the molecule"[8] in which sulfur and phosphorus may be split off in inorganic form,[141] ammonia[8, 227] and carbon dioxide[48] liberated, and ultimate hydrolysis products formed, while there still remains much material with properties very similar to those of the original casein[8] but differing in content of amino acids as revealed by determination of the nitrogen distribution.[109] In chemical hydrolysis of casein with 20 per cent hydrochloric acid the amino acid cystine is liberated rapidly, whereas in digestion with pepsin cystine is not liberated at all.[103] During digestion of casein with pepsin there is no consistent change in the total volume of the solution, but in hydrolysis in alkaline solutions there is a change in volume of 20 cubic millimeters per millimole of increase in free amino groups.[181] Enzymatic and chemical hydrolysis, therefore, follow distinctly different courses.

For purposes of studying its decomposition products (see Chapter 2) casein has been hydrolyzed in many ways including boiling in acid and alkaline solutions, heating with water under pressure,[109] and heating in alkaline solutions under pressure.[76] It has also been decomposed by treatment with anhydrous ammonia under pressure,[183] by heating with anhydrous glycerol or resorcinol,[99] and by heating in molten acetamide. In its technologic applications, however, we are concerned chiefly with minimizing the hydrolysis of casein or confining it to limits within which most of the casein remains in the form of the more complicated degradation products with properties similar to those of the initial casein.

Like many other proteins,[191, 206] casein probably is reasonably stable at room temperature between pH 2.5 and pH 8.5 only.[36, 86] Hydrolysis sets in more rapidly at higher temperatures,[222] while at lower temperatures the zone of pH in which there is reasonable stability is wider.[27] Above pH 10 or 10.5, a pH zone of great technologic importance, ammonia,[8, 227] sulfur and phosphorus[141, 226] are split off promptly. As would be expected from the opening up of free amino and carboxyl groups, the combining capacity for acids and bases increases during the hydrolysis of casein.[8, 178] In strongly alkaline solution the rate of hydrolysis is directly proportional to the activity of hydroxyl ions[19] and in acid solution to the activity of hydrogen ions.[146] The hydrolysis in acid solution is variously reported as a reaction of the first order[27] and as one of second order.[146]

In casein glues, paper coatings, and many casein paints it is usually necessary to work with casein in strongly alkaline solutions in which hydrolysis begins at once and progresses rather rapidly even at room temperature. Such solutions have a useful life of a few hours only; consequently the casein products must be handled and stored in dry form and mixed with water immediately before use. Casein paste paints of approximately neutral reaction and containing efficient antiseptics to prevent attack by microörganisms may be put up in prepared form and kept stable for months. In most casein products except casein plastics, however, the early hydrolytic products of casein rather than casein itself are the substances of which use is finally made.

The heat of combustion of casein is reported as 5.858 calories per gram.[184, p.84; 208, p.18]

References

1. Abderhalden, E. and Rossner, E., *Z. physik. Chem.*, **168**, 171 (1927)
2. Abramson, H. A., *Cold Spring Harbor Symposium Quant. Biology*, **1**, 39 (1933)
3. Almquist, H. J. and Greenberg, D. M., *J. Biol. Chem.*, **105**, 519 (1934)
4. Arthus, M. and Boshell, G., *Arch. intern. physiol.*, **30**, 244 (1928)
5. Atwood, F. C., U. S. Patent 1,893,608, Jan. 10, 1933
6. Bancroft, W. D., "Applied Colloid Chemistry," New York, McGraw-Hill Book Co., 1921
7. Bancroft, W. D. and Barnett, C. E., *J. Phys. Chem.*, **34**, 449 (1930); "Colloid Symposium Monograph," **7**, 151, New York, Reinhold Publishing Corp., 1929; *Proc. Natl. Acad. Sci.*, **16**, 118, 135 (1930)
8. Bancroft, W. D. and Browning, H. F., *J. Phys. Chem.*, **41**, 1163 (1937)
9. Bancroft, W. D. and Ridgway, S. L., *J. Phys. Chem.*, **36**, 1285 (1932)
10. Bartuszek, St., *Biochem. Z.*, **253**, 279 (1932)
11. Bilenskii, V. A. and Kastorskaya, T. L., *Colloid. J.* (U.S.S.R.), **2**, 193 (1936)
12. Bleyer, B. and Seidl, R., *Kolloid-Z.*, **30**, 117 (1922)
13. Bleyer, B. and Seidl, R., *Biochem. Z.*, **128**, 48 (1922)
14. Bosworth, A. W. and Van Slyke, L. L., *J. Biol. Chem.*, **14**, 207 (1913)
15. Bosworth, A. W. and Van Slyke, L. L., *J. Biol. Chem.*, **19**, 67 (1914)
16. Briggs, D. R., *J. Phys. Chem.*, **35**, 2914 (1931)
17. Brouse, D., *Ind. Eng. Chem.*, **21**, 242 (1929)
18. Browne, F. L. and Brouse, D., "Colloid Symposium Monograph," **5**, 229, New York, Reinhold Publishing Corp., 1928
19. Browne, F. L., Brouse, D. and Hrubesky, C. E., "Chem. and Phys. Studies of Water Resistant Glues," unpublished report of the U. S. Forest Products Laboratory, Jan. 26, 1929
20. Bruins, H. R., *Kolloid-Z.*, **57**, 152 (1931)
21. Bryson, H. C., *Brit. Plastics*, **3**, 411 (1932)
22. Buchner, E. H., *Rec. trav. chim.*, **51**, 619 (1932)
23. Burgess, L. L., *J. Am. Chem. Soc.*, **56**, 414 (1934)
24. Burk, N. F. and Greenberg, D. M., *J. Biol. Chem.*, **87**, 197 (1930)
25. Buzagh, A. v., *Kolloid-Z.*, **41**, 169; **43**, 215, 220 (1927)
26. Buzagh, A. v., *Kolloid-Z.*, **49**, 185 (1929)
27. Carpenter, D. C., *J. Biol. Chem.*, **67**, 647 (1926)
28. Carpenter, D. C., *J. Am. Chem. Soc.*, **57**, 129 (1935)
29. Carpenter, D. C. and Hucker, G. J., *J. Infectious Diseases*, **47**, 435 (1930)

30. Cherbuliez, E., *Compt. rend. soc. phys. hist. nat. Geneve,* **50,** 156 (1933), in *Arch. Sci. phys. nat.,* **15** (July-August 1933)
31. Cherbuliez, E. C. and Meyer, Fr., *Helv. Chim. Acta.,* **16,** 600 (1933)
32. Cherbuliez, E. C. and Schneider, M. L., *Helv. Chim. Acta.,* **15,** 597 (1932); *Lait,* **13,** 264 (1933)
33. Chick, H. and Martin, C. J., *Kolloid-Z.,* **11,** 102 (1912); **12,** 69 (1913)
34. Cohn, E. J., *J. Gen. Physiol.,* **4,** 697 (1922)
35. Cohn, E. J., *J. Biol. Chem.,* **59,** iv (1924)
36. Cohn, E. J. and Berggren, R. E. L., *J. Gen. Physiol.,* **7,** 45 (1924)
37. Cohn, E. J. and Conant, J. B., *Proc. Nat. Acad. Sci.,* **12,** 433 (1926); *Z. physiol. Chem.,* **159,** 93 (1926)
38. Cohn, E. J. and Hendry, J. L., *J. Gen. Physiol.,* **5,** 521 (1923)
39. Cohn, E. J., Hendry, J. L. and Prentiss, A. M., *J. Biol. Chem.,* **63,** 721 (1925); Cohn, E. J., *Physiol. Rev.,* **5,** 349 (1925)
40. Csonka, F. A. and Horn, M. J., *J. Biol. Chem.,* **93,** 677 (1931)
41. Czarnetsky, E. J. and Schmidt, C. L. A., *J. Biol. Chem.,* **105,** 301 (1934)
42. Damianovich, H. and Williams, A. T., *Anales soc. cient. Argentina,* **98,** 241 (1924)
43. Demanez, M. L., *Compt. rend. soc. biol.,* **112,** 1560 (1933)
44. Demanez, M. L., *Compt. rend. soc. biol.,* **112,** 1561 (1933)
45. Donnan, F. G., *Z. Elektrochem.,* **17,** 572 (1911)
46. Duclaux, E., "Le Lait Etudies Chimiques et Microbiologiques," Paris, 1889, cited by M. Beau, *Lait,* **12,** 618 (1932)
47. Dumanskii, A. and Puchkovskii, B. S., *Kolloid-Z.,* **48,** 338 (1929)
48. Dunn, M. S., *J. Am. Chem. Soc.,* **47,** 2564 (1925)
49. Ettisch, G. and Schulz, G., *Biochem. Z.,* **239,** 48 (1931); **245,** 189 (1932)
50. Euler, H. v. and Bucht, B., *Z. anorg. allgem. Chem.,* **126,** 269 (1923)
51. Faraday Society, "Colloidal Electrolytes," London, Gurney and Jackson, 1935
52. Fischenich, M. and Polanyi, M., *Kolloid-Z.,* **36,** 275 (1925)
53. Fischer, M. H. and Hooker, M. D., *Kolloid Beihefte,* **23,** 200 (1926)
54. Fischer, M. H. and Hooker, M. D., *Kolloid-Z.,* **47,** 193 (1929)
55. Fischer, M. H. and Hooker, M. D., *Kolloid Beihefte,* **40,** 241 (1934)
56. Fischer, M. H. and Sauer, W. J., *Arch. Path.,* **17,** 615 (1934)
57. Flexner L. B., *J. Biol. Chem.,* **121,** 615 (1937)
58. Fouassier, M., *Bull. soc. chim. biol.,* **5,** 487 (1923)
59. Fricke, R. and Lüke, J., *Naturwissenschaften,* **18,** 307 (1930); *Z. Elektrochem.,* **36,** 309 (1930)
60. Fürth, R., *Ann. Physik.,* **70,** 63 (1923)
61. Gahl, F., Greenberg, D. M. and Schmidt, C. L. A., *Univ. of Calif. Publications in Physiol.,* **5,** 307 (1926)
62. Gallay, W., *Can. J. Research,* **7,** 662 (1932)
63. Gorter, E. and Grendel, F., *Proc. Acad. Sci. Amsterdam,* **29,** 1262 (1926); *Trans. Faraday Soc.,* **22,** 477 (1926)
64. Gorter, E. and Seeder, W. A., *Kolloid-Z.,* **58,** 257 (1932)
65. Gould, S. P., *Ind. Eng. Chem.,* **24,** 1077 (1932)
66. Graham, T., *Phil. Trans.,* **151,** 183 (1861)
67. Green, A. A., *J. Biol. Chem.,* **93,** 517 (1931)
68. Greenberg, D. M., *Univ. Calif. Publications in Physiol.,* **7,** 9 (1927)
69. Greenberg, D. M., *Trans. Am. Electrochem. Soc.,* **54,** 107 (1928)
70. Greenberg, D. M. and Greenberg, M. M., *J. Biol. Chem.,* **94,** 373 (1931); *J. Gen. Physiol.,* **16,** 559 (1933)
71. Greenberg, D. M. and Larson, C. E., *J. Phys. Chem.,* **39,** 665 (1935)
72. Greenberg, D. M. and Schmidt, C. L. A., *Proc. Soc. Exptl. Biol. Med.* **21,** 281 (1924)

73. Greenberg, D. M. and Schmidt, C. L. A., *J. Gen. Physiol.*, **7**, 287 (1924)
74. Greenberg, D. M. and Schmidt, C. L. A., *J. Gen. Physiol.*, **7**, 303, 317 (1924)
75. Greenberg, D. M. and Schmidt, C. L. A., *J. Gen. Physiol.*, **8**, 271 (1926)
76. Griggs, M. A., *J. Ind. Eng. Chem.*, **13**, 1027 (1921)
77. Gróh, J., Karos, E., Dénes, K., and Serényi, V., *Z. physiol. Chem.*, **226**, 32 (1934)
78. Grynberg, M. D., *Biochem. Z.*, **262**, 272 (1933)
79. Haller, W., *Kolloid-Z.*, **56**, 170 (1931)
80. Haller, W., *Kolloid-Z.*, **57**, 197 (1931)
81. Hammarsten, O., *Z. physiol. Chem.*, **7**, 227 (1883)
82. Hewitt, L. F., *Biochem. J.*, **21**, 216 (1927)
83. Hiller, A. and Van Slyke, D. D., *J. Biol. Chem.*, **53**, 253 (1922)
84. Hitchcock, D. I., *J. Gen. Physiol.*, **5**, 383 (1923)
85. Hitchcock, D. I., *J. Gen. Physiol.*, **16**, 357 (1932)
86. Hoffman, W. F., *J. Biol. Chem.*, **65**, 251 (1925)
87. Hoffman, W. F. and Gortner, R. A., "Colloid Symposium Monograph," **2**, 209, New York, Reinhold Publishing Corp., 1924
88. Hoffman, W. F. and Gortner, R. A., *J. Phys. Chem.*. **29**, 769 (1925)
89. Holden, H. F. and Freeman, M. E., *Australian J. Exptl. Biol. Med. Sci.*, **7**, 13 (1930)
90. Holwerda, B. J., *Rec. trav. chim.*, **47**, 248 (1928)
91. Holwerda, B. J., *Rec. trav. chim.*. **50**, 601 (1931) ; *Verslag. landb. Onderzoek. Rijkslandbouwproefsta* No. 38C, 31 (1932)
92. Hoppe-Seyler, F., "Handb. d. physiol. und pathol. Chem.," 7th. ed., Berlin, 1903
93. Isgarischev, N. and Pomeranzeva, A., *Kolloid-Z.*, **38**, 235 (1926) ; *J. Russ. Phys. Chem. Soc.*, **58**, 164 (1926)
94. Isgarischev, N. and Pomeranzeva, A., *Kolloid-Z.*, **38**, 238 (1926) ; *J. Russ. Phys. Chem. Soc.*, **58**, 156 (1926)
95. Jirgensons, Br., *Biochem. Z.*, **195**, 134 (1928) ; *Kolloid-Z.*, **46**, 114 (1928)
96. Jirgensons, Br., *Biochem. Z.*, **240**, 218 (1931) ; *Kolloid-Z.*, **47**, 236 (1929)
97. Jirgensons, Br., *Kolloid-Z.*, **51**, 290 (1930)
98. Jirgensons, Br., *Kolloid-Z.*, **61**, 41 (1932)
99. Jirgensons, Br., *Biochem. Z.*, **246**, 219 (1932) ; **257**, 427 (1933)
100. Jirgensons, Br., *Biochem. Z.*, **268**, 414 (1934)
101. Jirgensons, Br., *Kolloid Beihefte*, **44**, 285 (1936)
102. Johlin, J. M., *J. Phys. Chem.*, **29**, 897 (1925)
103. Jones, D. B. and Gersdorff, C. E. F., *J. Biol. Chem.*, **101**, 657 (1933)
104. Katz, J. R., *K. Akad. Wetenschappen Amsterdam*, **13**, 958 (1910)
105. Katz, J. R., *Kolloidchem. Beihefte*, **9**, 1 (1917)
106. Katz, J. R., *Trans. Faraday Soc.*, **29**, 279 (1933)
107. Kikkoji, T., *Z. physiol. Chem.*, **61**, 139 (1909)
108. Kirk, P. L. and Schmidt, C. L. A., *J. Biol. Chem.*, **76**, 115 (1928)
109. Komatsu, S. and Okinaka, C., *Bull. Chem. Soc. Japan*, **1**, 102 (1926) ; *Mem. Coll. Sci. Kyoto Imp. Univ.*, **A10**, 249 (1927)
110. Kondo, K., *Compt. rend. trav. Lab. Carlsberg*, **15**, No. 8 (1925)
111. Kondo, K., and Hayashi, T., *Bull. Agr. Chem. Soc. Japan*, **2**, 146 (1926)
112. Kondo, K., and Hayashi, T., *Mem. Coll. Agr. Kyoto Imp. Univ.*, No. 5 (1928)
113. Kondo, K. and Tomiyama, S., *J. Chem. Soc. Japan*, **53**, 954 (1932)
114. Kondo, K. and Yamada, T., *J. Chem. Soc. Japan*, **53**, 898, 945 (1932)
115. Krishnan, R. S., *Proc. Indian Acad. Sci.*, **1A**, 717 (1935)
116. Kruyt, H. R., *Kolloid-Z. Special No.*, Apr. 1, 1925, p. 218
117. Kruyt, H. R., translated by Van Klooster, "Colloids," New York, John Wiley & Sons, 1927

118. Kruyt, H. R. and Lier, H., *Kolloid Beihefte*, **28**, 407 (1929)
119. Kruyt, H. R. and Tendeloos, H. J. C., *Kolloid Beihefte*, **29**, 413 (1929)
120. Laqueur, E. and Sackur, O., *Beit. chem. Physiol. Path.*, **3**, 193 (1902)
121. Levites, S. J., *Kolloid-Z.*, **8**, 4 (1910)
122. Lieben, F. and Löwe, L., *Biochem. Z.*, **252**, 64 (1932)
123. Linderstrøm-Lang, K., *Z. physiol. Chem.*, **176**, 76 (1928) ; *Compt. rend. trav. lab. Carlsberg*, **17** No. 9 (1929)
124. Linderstrøm-Lang, K. and Kodama, S., *Compt. rend. trav. lab. Carlsberg*, **16** No. 1, 1 (1925)
125. Linderstrøm-Lang, K. and Kodama, S., *Comp. rend. trav. lab. Carlsberg*, **16** No. 1, 48 (1925)
126. Lindet, L., *Lait*, **1**, 161 (1921)
127. Lisitzuin, M. A., *Biochem. Z.*, **266**, 25 (1933)
128. Loeb, J., "Proteins and the Theory of Colloidal Behavior," New York, McGraw-Hill Book Co., 1922
129. Loeb, J., *J. Gen. Physiol.*, **1**, 39, 237 (1918-19) ; *Science*, **52**, 449 (1920) ; *J. chim. phys.*, **18**, 283 (1920)
130. Loeb, J., *J. Gen. Physiol.*, **3**, 547 (1920-21)
131. Loeb, J., *J. Gen. Physiol.*, **4**, 73 (1921-22)
132. Loeb, J., *J. Am. Chem. Soc.*, **44**, 1930 (1922)
133. Loeb, J., *J. Gen. Physiol.*, **5**, 395 (1923)
134. Loeb, J. and Loeb, R. F., *J. Gen. Physiol.*, **4**, 187 (1921-22)
135. Loiseleur, J., *Bull. soc. chim. biol.*, **14**, 1088 (1932) ; *Compt. rend.*, **191**, 1477 (1930)
136. Loiseleur, J. and Velluz, L., *Compt. rend.*, **192**, 159 (1931)
137. Long, J. H., *J. Am. Chem. Soc.*, **27**, 363 (1905)
138. Lottermoser, A. and Calantar, N., *Kolloid-Z.*, **48**, 362 (1929)
139. Mandoki, L. and Polanyi, M., *Biochem. Z.*, **104**, 254 (1920)
140. Marchlewski, L. and Wjerzuchowska, J., *Bull. intern. de l'acad. Pol. Sci.*, **1928A**, 471; *Chem. Abstracts*, **20**, 2147
141. Maynard, L. A., *J. Phys. Chem.*, **23**, 145 (1919)
141a. McKee, R. H. and Gould, S. P., *J. of Agric. Research*, **57**, 125 (1938)
142. Mellanby, J., *Proc. Physiol. Soc.*, in *J. Physiol.*, **54**, cxx (1920)
143. Michaelis, L. and Szent-Györgyi, A. v., *Biochem. Z.*, **103**, 178 (1920)
144. Miyomoto, S. and Schmidt, C. L. A., *J. Biol. Chem.*, **99**, 335 (1933)
145. Mohr, W. and Moos, J., *Milchwirtschaft. Forsch.*, **15**, 384 (1933)
146. Nasset, E. S. and Greenberg, D. M., *J. Am. Chem. Soc.*, **51**, 836 (1929)
147. Negri, E., *Arch. farmacol. sper.*, **53**, 72 (1931)
148. Nicolas, E., *Lait*, **12**, 593 (1932)
149. Osborne, T. B. and Wakeman, A. J., *J. Biol. Chem.*, **33**, 243 (1918)
150. Osborne, W. A., *J. of Physiol.*, **27**, 398 (1901)
151. Ostwald, Wo., *Kolloid-Z.*, **41**, 163; **43**, 249 (1927)
152. Pauli, Wo., *Biochem. Z.*, **70**, 489 (1915)
153. Pauli, Wo., and Matula, J. M., *Biochem. Z.*, **99**, 219 (1919)
154. Pauli, Wo., *Biochem. Z.*, **127**, 150 (1922)
155. Pauli, Wo., "Colloid Chemistry of the Proteins," Churchill, London, 1922
156. Pauli, Wo. and Hofmann, L., *Kolloid Beihefte*, **42**, 34 (1935)
157. Pauli, Wo. and Matula, J. M., *Biochem. Z.*, **80**, 187 (1917)
158. Perov, S. S., *Trans. Volgoda Dairy Institute, Bull. No. 50* (1925) ; *Chem. Abstracts*, **22**, 787
159. Pertzoff, V., *J. Gen. Physiol.*, **10**, 961 (1927)
160. Pertzoff, V., *J. Gen. Physiol.*, **10**, 987 (1927) ; **11**, 239 (1928)
161. Pertzoff, V., *J. Biol. Chem.*, **79**, 799 (1928)
162. Pertzoff, V. and Bell, B., *Lait*, **12**, 161 (1932)

163. Pertzoff, V. and Carpenter, S. C., *J. Gen. Physiol.*, **16**, 257 (1932)
164. Petrescu, V., *Ann. Sci. univ. Jassy*, **18**, 318 (1933); *Chem. Abstracts*, **27**, 2613
165. Porcher, Ch., *Chimie & industrie*, **19**, 589, 809 (1928)
165a. Popkov, S., *Org. Chem. Ind.* (U.S.S.R.), **5**, 27 (1938)
166. Prideaux, E. B. R. and Howitt, F. O., *Proc. Roy. Soc. (London)*, **A126**, 126 (1929)
167. Proctor, H. R. and Wilson, J. A., *J. Chem. Soc.*, **109**, 307 (1916)
168. Rakusin, M. A., *J. Russ. Phys. Chem. Soc.*, **47**, 147 (1915); *Chem. Abstracts*, **9**, 1488
169. Rakusin, M. A., *Biochem. Z.*, **192**, 167 (1928)
170. Rawlins, L. M. C., and Schmidt, C. L. A., *J. Biol. Chem.*, **82**, 709 (1929)
171. Robertson, T. B., *J. Biol. Chem.*, **2**, 317 (1907)
172. Robertson, T. B., *J. Phys. Chem.*, **13**, 469 (1909)
173. Robertson, T. B., *J. Phys. Chem.*, **14**, 377 (1910); Robertson and Migake, *J. Biol. Chem.*, **25**, 351 (1916)
174. Robertson, T. B., *J. Phys. Chem.*, **14**, 528 (1910)
175. Robertson, T. B., *J. Biol. Chem.*, **8**, 507 (1910)
176. Robertson, T. B., *J. Phys. Chem.*, **15**, 179 (1911)
177. Robertson, T. B., *J. Phys. Chem.*, **15**, 387 (1911)
178. Robertson, T. B., *Australian J. Exptl. Biol.*, **1**, 31 (1924)
179. Robertson, T. B., "Physical Chemistry of the Proteins," New York, Longmans Green, 1918
180. Robinson, A. D., Gortner, R. A. and Palmer, L. S., *J. Phys. Chem.*, **36**, 1857 (1932)
181. Rona, P. and Fischgold, H., *Biochem. Z.*, **261**, 366 (1933)
182. Ryd, S., *Z. Elektrochem.*, **23**, 19 (1917); *J. Soc. Chem. Ind.*, **36**, 561 (1917)
183. Sadikov, V. S., *Biochem. Z.*, **205**, 360 (1929)
184. Scherer, R., translated by H. B. Stocks, "Casein, Its Preparation and Technical Utilisation," 3d. ed., New York, D. Van Nostrand Co., Inc., 1921
185. Schmidt, C. L. A., *J. Biol. Chem.*, **23**, 487 (1915)
186. Schmitz, Ad., *Z. physiol. Chem.*, **221**, 197 (1933)
187. Schryver, S. B., *Proc. Roy. Soc. (London)*, **B86**, 460 (1913)
188. Seifriz, E., *J. Phys. Chem.*, **29**, 587, 595, 738 (1925)
189. Seifriz, E., *J. Phys. Chem.*, **29**, 834 (1925)
190. Selmi, F., *Nuovi Ann. di Scienze Natur. Bologna*, (2) **8**, 404 (1847): cf. *Kolloid-Z.*, **8**, 113 (1911)
191. Sjögren, B. and Svedberg, T., *J. Am. Chem. Soc.*, **52**, 5187 (1930)
192. Smoluchowski, V., *Kolloid-Z.*, **18**, 190 (1916)
193. Sørensen, S. P. L., *Compt. rend. trav. lab. Carlsberg*, **15** No. 1 (1923)
194. Sørensen, S. P. L., *J. Am. Chem. Soc.*, **47**, 457 (1925)
195. Sørensen, S. P. L., *Kolloid-Z.*, **53**, 102 (1930)
196. Sørensen, S. P. L., *Kolloid-Z.*, **53**, 170 (1930)
197. Sørensen, S. P. L. and Sladek, I., *Compt. rend. trav. lab. Carlsberg*, **17** No. 14 (1929); *Kolloid-Z.*, **49**, 16 (1929); Sladek, *Casopis Ceskoslov. Lekarnictva*, **10**, 1, 29, 61 (1930)
198. Stamm, A. J. and Loughborough, W. K., *J. Phys. Chem.*, **39**, 121 (1935)
199. Starlinger, W., *Biochem. Z.*, **170**, 1 (1926)
200. Stocks, H. B., *J. Intern. Soc. Leather Trades Chem.*, **10**, 409 (1926)
201. Straub, J., *Rec. trav. chim.*, **45**, 535 (1926)
202. Subbaramaiya, D. S., *Proc. Indian Acad. Sci.*, **1A**, 709 (1935)
203. Svedberg, T., "Colloid Chemistry," 2d. ed., New York, Reinhold Publishing Corp., 1928

204. Svedberg, T., *Nature*, **123**, 871 (1929)
205. Svedberg, T., *Kolloid-Z.*, **51**, 10 (1930)
206. Svedberg, T., *Trans. Faraday Soc.*, **26**, 740 (1930)
207. Svedberg, T., Carpenter, L. M. and Carpenter, D. C., *J. Am. Chem. Soc.*, **52**, 241, 701 (1930); Carpenter, D. C., *J. Am. Chem. Soc.*, **53**, 1812 (1931)
208. Tague, E. L., "Casein, Its Preparation, Chemistry and Technical Utilization," New York, D. Van Nostrand Co., Inc., 1926
209. Tsett, *Bull. soc. chim.*, (3) **23**, 309 (1900); *Compt. rend.*, **129**, 551 (1899)
210. Van Bemmelen, J. M., *Z. anorg. Chem.*, **5**, 466 (1894); **13**, 233 (1897); **30**, 265 (1902)
211. Van Slyke, L. L. and Bosworth, A. W., *J. Biol. Chem.*, **14**, 211, 227 (1913)
212. Van Slyke, L. L. and Hart, E. B., *Am. Chem. J.*, **33**, 461 (1905)
213. Van Slyke, L. L. and Van Slyke, D. D., *Am. Chem. J.*, **38**, 383 (1907); *J. Biol. Chem.*, **4**, 259 (1908); *N. Y. Agr. Expt. Sta. (Geneva) Tech. Bull., No. 3* (Dec. 1906)
214. Van Slyke, L. L. and Winter, O. B., *Science*, **38**, 639 (1913)
215. Van Slyke, L. L. and Winter, O. B., *J. Biol. Chem.*, **17**, 287 (1914)
216. Veimarn, P. P. v., *J. Textile Inst.*, **17**, T642 (1926); *Can. Chem. Met.*, **10**, 227 (1926); *Ind. Eng. Chem.*, **19**, 109 (1927)
217. Veimarn, P. P. v., *Japan J. Chem.*, **3**, 71 (1928); *Kolloid-Z.*, **42**, 134 (1927)
218. Wagner, H., *Kolloid-Z.*, **47**, 19 (1929)
219. Wagner, H. and Kesselring, J., *Farben. Ztg.*, **34**, 947 (1929)
220. Wilson, J. A., *J. Am. Chem. Soc.*, **38**, 1982 (1916)
221. Wright, N. C., *J. Agr. Sci.*, **16**, 640 (1926)
222. Yaichnikov, I. S., *J. Russ. Phys. Chem. Soc.*, **54**, 814 (1924); **58**, 1374 (1926)
223. Yamakami, K., *Biochem. J.*, **14**, 522 (1920)
224. Zaykovskii, I., *Biochem. Z.*, **137**, 562 (1923)
225. Zeldin, S., *Malyarnoe Delo*, **1932** No. 10, 13; *Chem. Zentr.*, **1933I**, 3857
226. Zoller, H. F., "The Theory and Application of Colloidal Behavior" by R. H. Bogue, ch. 33, New York, McGraw-Hill Book Co., 1924
227. Zoller, H. F., *Science*, **50**, 49 (1919); *J. Gen. Physiol.*, **3**, 635 (1921)
228. Zsigmondy, R., *Z. anal. Chem.*, **40**, 697 (1901)
229. Zsigmondy, R., *Z. anorg. Chem.*, **71**, 356 (1911); *Physik. Z.*, **14**, 1098 (1913)

Additional References

230. Bell, R. W. and Gould, S. P., *J. Dairy Sci.*, **14**, 337 (1931)
231. Gortner, R. A. and Hoffman, W. L., *J. Phys. Chem.*, **31**, 464 (1927)
232. Kruyt, H. R., *Bull. soc. chim.*, (5) **4**, 1925 (1937)
233. Neuberger, A., *Proc. Roy. Soc. (London)*, **A158**, 68 (1937)
234. Olsen, A. G., *J. Phys. Chem.*, **36**, 529 (1932)
235. Schmid, G. and Erkkila, A. V., *Z. Elektrochem.*, **42**, 737, 781 (1936); Schmid and Aalto, T. R., *Ibid.*, **43**, 907 (1937)
236. Stone, G. C. H. and Failey, C. F., *J. Phys. Chem.*, **37**, 935 (1933); also Failey, *Ibid.*, 1075; *J. Am. Chem. Soc.*, **54**, 576, 2367 (1932); **55**, 4374 (1933); and Adair, G. S., *Proc. Roy. Soc. (London)*, **A120**, 573 (1928)
237. Taylor, H. S., "A Treatise on Physical Chemistry," 2d. ed., New York, D. Van Nostrand Co., Inc., 1930
238. Cheesman, D. F. and King, A., *Trans. Faraday Soc.*, **34**, 594 (1938)
239. Leichter, H., Umbach, G., and Nord, F. F., *Biochem. Z.*, **291**, 191 (1937)
240. Nitschmann, H., *Helv. Chim. Acta.*, **21**, 315 (1938)
241. Porcher, Ch., Volkringer, H. and Brigando, J., *Compt. rend.*, **199**, 249 (1934)
242. Schaefer, V. J., *J. Phys. Chem.*, **42**, 1089 (1938)

Chapter 4

The Manufacture of Casein

Arnold O. Dahlberg

Only a small portion of the skim milk produced in the creameries of the United States finds its way into the manufacture of casein. Most of it is required for foodstuffs or for feeding animals. When the first edition of this book appeared in 1927 casein was largely a seasonal by-product during a few months of the summer when creameries had a surplus of skim milk. The producers of casein for the most part made it on a small scale and chose their methods for simplicity, convenience, and low capital investment in equipment rather than for production of casein of uniformly high quality. Many manufacturing procedures were in use, most of them of long standing and ill adapted to precise control. The resultant variation in quality and properties of casein seriously hampered the industries using it and led to frequent preference for imported casein, which was found to be more uniform. Between 1916 and 1928 domestic production exceeded importation of casein only in 1919 and 1924 (see Chapter 13).

In the first edition of this book it was pointed out that the general quality of domestic casein could be improved through standardization of manufacturing methods and adoption of improved procedures developed by the Bureau of Dairying of the U. S. Department of Agriculture[11, 12, 39, 40, 47] and by state agricultural experiment stations.[31, 41, 43a] In the intervening time there has been marked improvement in the casein made by many of the small producers. There has also been a material increase in production of casein by large plants having new and more expensive equipment, closer technical supervision, and more definite commitment to supply a product of uniform properties and good quality to meet their customers' requirements. Since 1929 domestic production of casein has always exceeded importation and in spite of business depression has remained greater than it ever was before 1929. To be sure an increase in the tariff on imported casein from 2.5 to 5.5 cents a pound since June, 1930 has played a part in the dwindling importation but improvement in the domestic product has been an important factor as well.

The new equipment and improved methods of manufacturing casein

are based upon more thorough understanding of the technical principles involved. Reduction in the cost of manufacturing has accompanied the improvement of the product. The main changes in practice are:

1. More general use of methods giving a granular curd from which impurities are easily washed, thus yielding casein containing less acid and ash.
2. Development of continuous methods of precipitation.
3. Elimination of the old hand process of pressing the wet curd by producing curd that drains well enough to be ground without pressing.
4. Quicker drying by securing a curd that withstands higher temperatures in the tunnel drier, or by using a continuous drier.

Availability of casein of uniformly high quality makes it increasingly difficult to market the poorer grades produced in small plants following old methods with little attention to standardization and control. Establishment of a central plant with modern methods and proper control of manufacture is the logical way to improve the quality of casein in a territory in which several small plants operating independently make variable products.

Casein manufacture is sometimes considered impracticable in a plant making other dairy products because of the objectionable odors formerly believed a necessary concomitant of the process. Although casein too often is made under such conditions, objectionable odors are by no means necessary. With proper equipment and exercise of reasonable care commercial casein can be made without danger of impairing the odor or flavor of other dairy products.

The quality of casein depends upon the skim milk from which it is made, the method of precipitation, the thoroughness with which the curd is washed, the temperature and time of drying, and other conditions of the manufacturing operations. The first essential for making casein of good quality is skim milk of centrifugal separation, low in fat and free from foam at the time of precipitating the curd. Foam on skim milk when the curd is precipitated gives trouble by forming insoluble particles in the dry casein.*

Equipment Required

The principal equipment required for making casein from skim milk by discontinuous processes includes the following:[40]

* As pointed out in Chapter 3, casein concentrates in multimolecular layers at the surface of solutions of the acidity of whey. The casein in such surface films is denatured and insoluble. Foam enormously increases the area of solution-air interface and therefore the amount of casein denatured in this way.

1. Storage vats for skim milk and whey.
2. Precipitating vats or other equipment for precipitating the curd.
3. Curd presses, if the method of precipitation yields a curd that does not drain readily. Presses may be either manually operated with dividing boards and press cloths or continuously operated by power.
4. Drain vats or racks, particularly in methods that eliminate presses.
5. Suitable curd grinder.
6. Drier with steam coils and low-pressure fan blower for circulation of air. Either a tunnel drier requiring drying trays covered with screens and trucks or a continuous drier may be used.

Where cooked curd is shipped wet to a central drying plant the only equipment required is the precipitating vat. Dealers in casein can furnish the equipment necessary for making casein by intermittent methods and can give reliable information for its installation.

Precipitating Vats

Wooden vats are largely used because they are cheap and withstand acids. Redwood, cypress, Douglas fir, and southern yellow pine are widely used. Redwood and cypress, if of a grade containing heartwood only, are more resistant to decay but are softer and less resistant to mechanical wear than Douglas fir or southern yellow pine. Iron vats are also in use although they are subject to corrosion.[22]

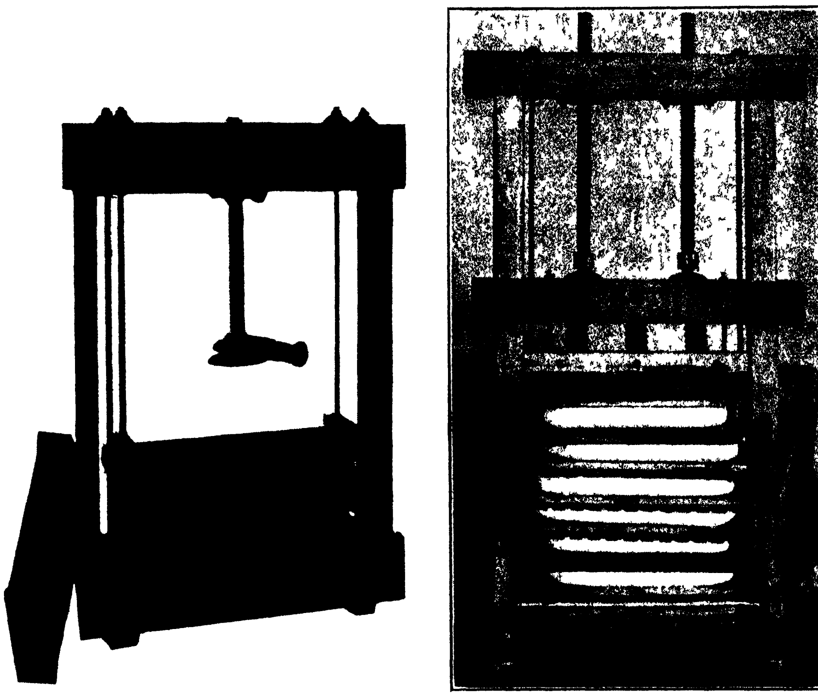
Rectangular vats made like cheese vats with the bottom sloping and a gate valve at the low end for draining the whey, and holding from 6,000 to 8,000 pounds of skim milk are satisfactory for handling small quantities of skim milk. Wooden vats should be made of 2-inch stock and braced with rods or long bolts under the bottom and along the sides. For larger quantities of skim milk larger vats with round bottoms, provided with mechanical agitators (Figure 16) reduce the labor cost, allow quicker handling of the skim milk, and provide better control of quality. The larger agitator vat lends itself well to the continuous precipitation method of collecting and washing the curd.

In the Sheffield and the Spellacy continuous processes, which are based upon specially designed precipitating machines, no vats are required except for storage of skim milk. Precipitation takes place continuously in a small earthenware box containing baffles or in a T-tube mixer made of acid-proof material.

Curd Presses, Manually Operated Type

Curd presses of the old, manually operated type, with dividing boards and press cloths, as shown in Figure 8, are still used a great deal al-

though they are being displaced by processes in which pressing is unnecessary or, among the large manufacturers, by continuous power presses. Curd presses of the hand type must be well made to stand the heavy strain to which they are subjected, and should be so constructed that they can be kept clean easily. They are usually purchased ready-made but may be made at the plant if desired.



Courtesy of R A Simpson Mfg Co *Courtesy of U S Department of Agriculture*

Figure 8.—Curd presses with dividing and press boards The press at the left is empty, that at the right, which is equipped with springs, contains curd under pressure.

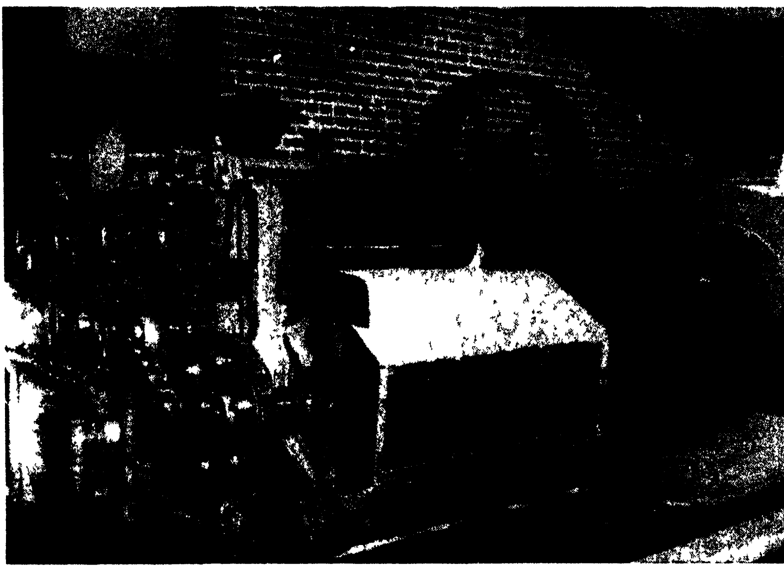
The dividing boards for use between bundles of wet curd consist of hardwood strips ½ by 1⅜ by 30 inches in size nailed crosswise, making a slatted form 30 inches square with approximately one-inch spaces between strips. The boards should be given two coats of linseed oil or penetrating floor seal to retard the absorption of moisture while in use. They should be washed daily with hot water and a brush to prevent offensive odors.

Press cloths are used to wrap wet curd into bundles to be placed be-

tween the dividing boards in hand presses. Burlap sacking is often used but it is difficult to wash and keep clean. Ten-ounce duck is very satisfactory because it is strong, easy to wash, and permits none of the curd to escape. The cut ends of the cloths should be hemmed. Two cloths are generally used for each bundle of curd.

Curd Presses, Continuous Type

Continuous presses such as those shown in Figures 9 and 21 reduce the moisture content of the curd to about 55 per cent by passing it between revolving rolls. The upper roll is solid and by the pressure of a



Courtesy of Louisville Drying Machinery Co.

Figure 9—An eight-roll, power driven, continuous curd press.

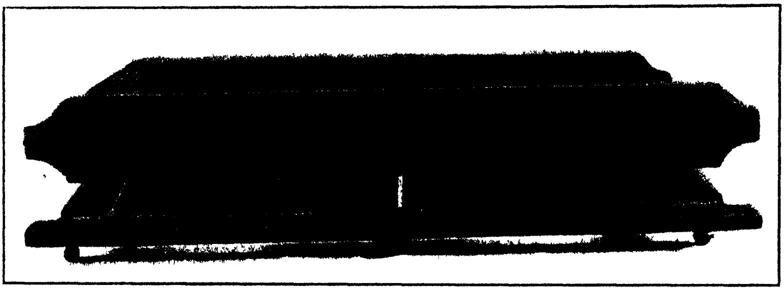
spring forces the curd against the lower perforated roll or screen to squeeze out the free water. Continuous presses permit the precipitated curd to be placed in the drier in much less time than is required by the old press with dividing boards. The capacity of continuous presses ranges from 1,000 to 2,000 pounds of wet curd, equivalent to 500 to 1,000 pounds of dry casein per hour.

Drain Vats and Drain Racks

Methods that produce a curd capable of draining well enough for grinding without pressing require a drain vat like that shown in Figure

16 or drain racks such as the one shown in Figure 10. The drain vat is built much like a precipitating vat except that it has a false bottom of perforated sheet iron through which the whey and wash water drain, leaving the curd behind.

Drain racks should be made of cypress or redwood heartwood for lightness and ability to stand moisture. The one shown in Figure 10 consists of two parts, the lower part a frame of 2- by 4-inch wood 3 feet 4 inches wide by 6 feet 4 inches long, over which is nailed galvanized wire screening of 4 meshes per inch. The screen is further supported by lengths of ½-inch galvanized iron pipe running across the frame at intervals of one foot and sunk into the 2- by 4-inch



Courtesy of U. S. Department of Agriculture

Figure 10.—A drain rack on casters for convenience in moving. The drain cloth is not shown.

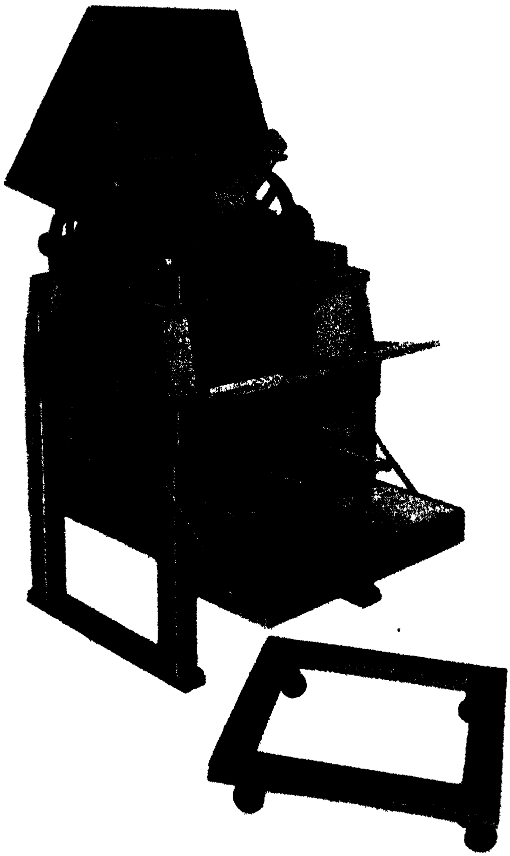
members. The frame rests on galvanized iron castors for ease in moving it about. The upper part of the rack is made of 1½- by 12-inch boards.

A drain cloth is required to cover the rack and filter off the casein. A coarse weave marquisette or scrim is excellent because it is strong, closely woven, permits easy drainage, and is easy to wash. Burlap is serviceable but is harder to wash and may not allow such good drainage. The drain cloth should be made large enough to extend over the sides and ends of the drain rack and the cut edges should be hemmed to prevent raveling.

Two drain racks are enough to handle the curd from 5,000 pounds of skim milk. The curd drains quickly so that the racks can be used alternately until the batch is finished. One drain rack is often of advantage in methods in which they are not regularly employed because at times conditions are encountered that give much fine curd, most of which may be lost in the whey if draining facilities of ample capacity are not available.

Curd Mill

The right kind of mill for grinding the curd is important because correctly ground curd is essential for attaining favorable drying conditions.

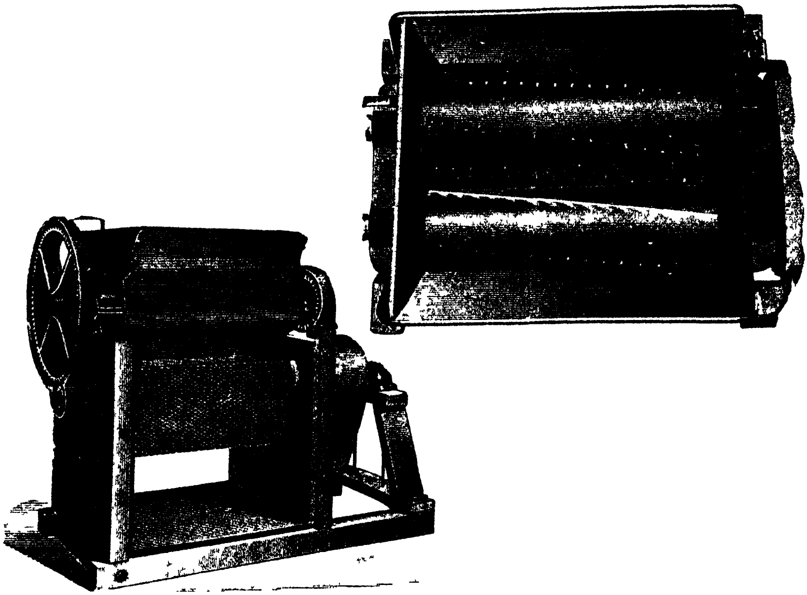


Courtesy of R. A. Simpson Co.

Figure 11.—A curd mill with arrangement for spreading the curd directly on trays after grinding.

The mill should have both a shredding and a beating action. In the better machines (Figure 11) the shredding is accomplished by a cylinder revolving comparatively slowly and fitted with sharp peg teeth that tear the curd into pieces. The pieces fall into a lower cylindrical drum of heavy, perforated metal in which a rapidly revolving iron beater drives the curd through the perforations. Especially strong mills are

required for cooked curds (Figure 12) because they are very tough and hard to grind.



Courtesy of Walton Foundry Co.

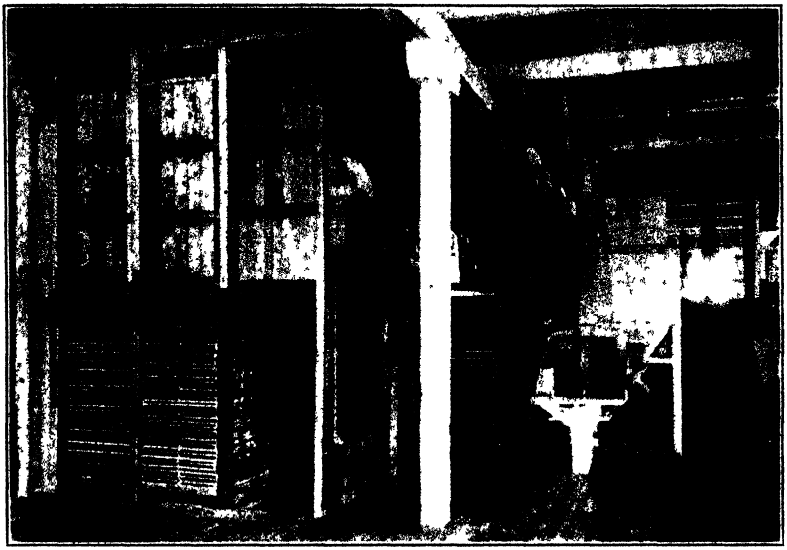
Figure 12—A curd mill suitable for grinding cooked curd

Tunnel Driers

The tunnel drier is a chamber of wooden construction about 22 feet long, 5 or 6 feet high and 31 or 32 inches wide, through which trays of curd stacked on trucks can be rolled against a current of heated air (Figure 13). The inside of the tunnel may be lined with galvanized iron. Two or more driers may be built side by side each sharing a wall in common for economy in construction and floor space and to reduce heat losses. The ends of the tunnel are provided with vertical sliding doors. The walls should be well insulated to conserve heat. Truck loads of drying trays holding wet curd are pushed in at one end of the tunnel and withdrawn at the other when the casein is dry. A double tunnel of the above dimensions when filled with drying trays holds the casein from 30,000 pounds of skim milk.

A low-pressure blower of fan type is used for circulating the heated air through the driers. It should be of sufficient size to provide the required volume of heated air and connected with steam heating coils of ample radiating surface to heat the air to the temperature desired. With a drying temperature of 120° to 135°F. in tunnel driers, it is

recommended that air be supplied at the rate of four cubic feet per minute per pound of wet curd per hour going into the drier.[38] It is advisable to have the blower and heating coils somewhat over-size and to regulate the volume of air with dampers. Reliable information on the size of blower and heating coils may be obtained from the manufacturers of this equipment by furnishing them with data on the conditions and quantity of curd to be dried in a given time.



Courtesy of Golden State Co.

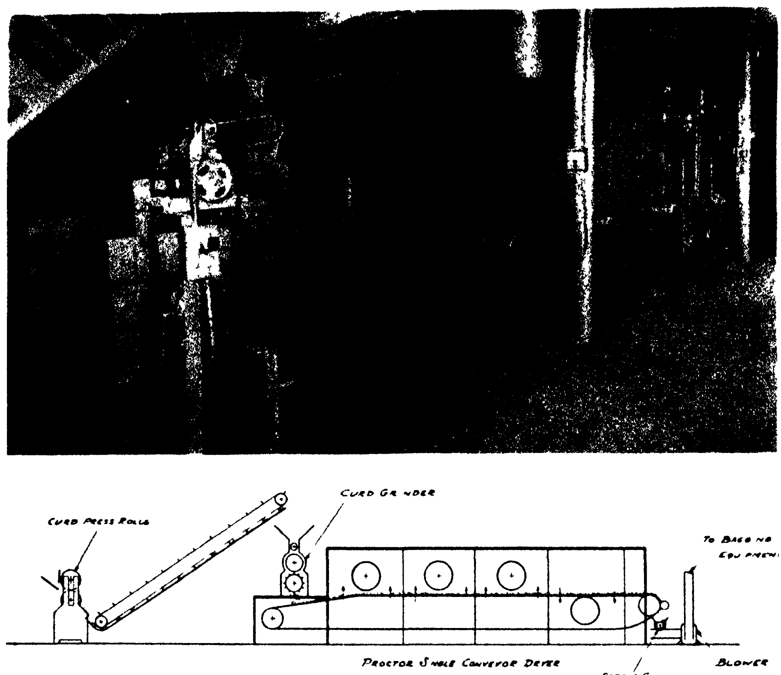
Figure 13.—A tunnel drier with three tunnels, showing trays on trucks in two of the tunnels. Fan blower and heating coils are on top of the drier.

Drying trays 30 by 30 inches in size can be purchased ready-made. They consist of a wooden or metal frame covered with heavy galvanized screening of 14 to 16 meshes per inch. The trucks are likewise 30 by 30 inches in size with heavy wooden frame provided with a swivel caster at each corner. The trays carrying the wet curd neatly spread in layers of proper thickness are stacked one above another on the trucks to be rolled into the drier. Each tray should carry the equivalent of 1½ to 2 pounds of dried casein. Twelve trucks and 30 dozen trays are required to handle the curd from 20,000 pounds of skim milk daily.

Continuous Driers

The tunnel driers have the advantage of low initial cost of the equipment but, for large plants at least, the continuous driers prove more economical in operation and turn out a superior product. Since drying

is the most expensive step in making casein, economy in operation of the drier is very important. The continuous driers effect savings in labor, fuel, floor space, and upkeep of the trays and trucks they displace and in wear on the flooring of the building, which becomes an item of consequence where trucks must be rolled constantly. Improvement in the color and general quality of the casein is effected by decreasing



Courtesy of Proctor and Schwartz, Inc.

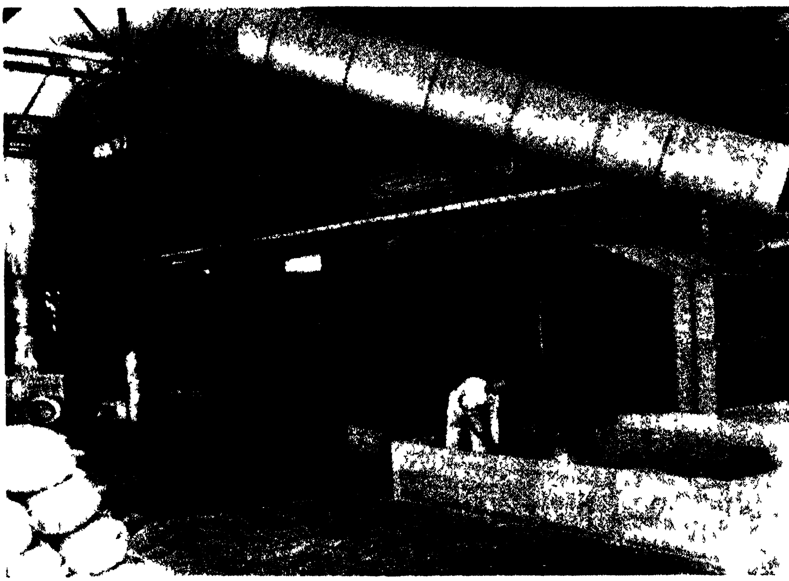
Figure 14.—A continuous wire screen conveyor drier.

the time between pressing and final drying and largely eliminating putrefaction.

There are two types of continuous drier on the market, the sectional wire screen conveyor type and the revolving drum type.

The sectional wire screen conveyor drier (Figure 14) is a traveling screen conveyor made in sections to the length desired for capacities of 215 pounds or more of dried casein per hour, handling curd containing 53 to 60 per cent moisture. The conveyor, made of sections of fine screen joined together by special lacing similar to that used on belts, is carried through a drying enclosure in which heated air is circulated up and down through the layer of evenly spread curd. The ground curd

is automatically spread on the screen conveyor at one end and automatically discharged as dry casein at the other end in 18 to 40 minutes. The drying enclosure is divided into three compartments of which the first and third operate at 130°F. and the second at 200°F. The lower temperature at the beginning keeps the surface from hardening and leaves the curd in favorable condition for rapid drying in the second compartment.



Courtesy of Louisville Drying Machinery Co.

Figure 15—A rotary continuous drier

The rotary drier (Figure 15) consists of a long, cylindrical, iron drum, lying nearly horizontal and revolving slowly. Heated air is passed through the drum counter-current to the movement of the curd. The pressed or de-watered curd is fed automatically into one end of the drier and, while slowly passing through it, is repeatedly lifted to the top of the drum and dropped in a shower through the heated air. The end at which the dry casein is automatically discharged is kept at 160 to 170°F., while the end at which the curd enters is about 120 to 130°F. Approximately three hours is required for the curd to travel through the drier. Because of the intimate contact between curd and air the thermal efficiency of this drier is said to be high.

In addition to the two continuous driers described a third one has been developed in which the curd is carried through pipe conveyors by a current of heated air.[9] It is claimed that drying is completed in 45

to 60 seconds, that all danger of decomposition of moist curd is removed, and that the casein produced makes mixtures for coating paper in which the tendency to foaming is reduced. There is also a patent on the spray-drying of casein.[30]

Unit Processes in Casein Manufacture

Casein is made by a number of methods that differ in important details but make common use of certain general procedures. For that reason it is convenient to divide the manufacturing operations into six unit processes and to discuss the unit processes before describing their application in the several manufacturing methods. General principles of the isolation of casein from milk and the differences in the caseins produced by the different methods are discussed in Chapter 1.

The unit processes in the manufacture of casein are: precipitation, washing, de-watering, grinding the curd, drying, and grinding and packing the product. The operations are performed in the order named.

Precipitation

Most commercial casein is precipitated from skim milk by the action of an acid, either hydrochloric acid or sulfuric acid added to the skim milk or lactic acid produced by fermentation of the lactose when the milk sours. A smaller quantity of commercial casein is precipitated by the action of rennet. Choice of reagent may be dictated by the manufacturer's customers; the plastics industry, for example, usually demands rennet casein, but the paint, glue, or paper maker as a rule refuses to buy it. If milk sugar is to be recovered from the whey left after precipitating the casein, hydrochloric acid is the preferred reagent because sulfuric acid leaves calcium sulfate in the whey. The limited solubility of calcium sulfate complicates the separation of milk sugar, and lactic acid, of course, is formed at the expense of the milk sugar subsequently obtainable.[23] The whey from rennet coagulation can also be used satisfactorily for making milk sugar.

Precise control of the precipitation of the curd is vital for economical production of casein of high quality. Only when the curd is precipitated properly can it be readily washed free from excess acid and constituents of the whey.[5, 11, 31, 40, 41] In commercial production of casein considerations of cost prohibit resolution of the curd and reprecipitation as is generally done in laboratory methods.

For reasons pointed out in Chapter 1 the most important factor in precipitating the curd is the intensity of acidity, or pH, at which the separation of curd from whey takes place. At pH 4.6 casein is freed from combination with calcium phosphate, reaches its minimum solubility, and the curd is of the granular type most easily washed free of contaminants. According to Trimble and Bell[40] most manufacturing

methods require that the pH be brought beyond the isoelectric point, approximately to pH 4.1, in order to obtain a curd that is porous, resilient, and granular and retains these properties during washing and handling. The end point must be determined by exact methods as well as by physical examination of the curd.

Quoting from Trimble and Bell:[40] "The amount of acid necessary to attain this end point, at which the most desirable type of curd forms, depends on such facto s as the initial acidity of the skim milk, the composition of the skim milk, the precipitation temperature, kind and dilution of acid, methods of adding and mixing the acid with the skim milk, and methods of handling the precipitated curd. The variety of these factors indicates the need of using exact methods for attaining the optimum end point. For example, if the precipitating temperature is high, a clear whey will be obtained after a relatively small amount of acid has been added. The curd can be easily drained but will not be porous, resilient, or granular and, therefore, cannot be effectively washed. Either insufficient acid or an excess of acid is detrimental to the production of curd that will make a finished casein of the highest quality.

"In practice the quickest and most satisfactory method for determining when the desired acidity of the casein whey has been reached is in the use of bromocresol green indicator paper."[3, 44] "A strip of the paper is dipped in the whey and placed on a window pane not in direct sunlight. Its color viewed by transmitted light is then compared with a standard set of colors viewed by reflected light. The standard colors correspond to pH values within the acidity range near the desired end point. The observation must be made quickly, as the color of the wet indicator paper changes on standing." "After some practice with this method an operator can gage the end point reached within 0.1 pH unit of the actual pH value of the whey as determined by a potentiometric titration. Values ranging from approximately pH 3.8 to 5.2 can be determined by this method. The color gradations which correspond to these values range from a bright yellow (pH 3.8 and below) to a deep blue (pH 5.2 and above). The paper is greenish yellow if the acidity of the whey is at or near pH 4.1, the most desirable end point. Deeper shades of green gradually turning to blue indicate higher (less acid) pH values. Colored paper standards and bromocresol green indicator paper are commercially available. The color standards should be used only for comparative purposes.

"Bromocresol green paper can also be used to determine the acid intensity of the wash waters. When the water imparts a green or yellowish-green color to the paper (pH 4.3 to 4.6), it is sufficiently acid to use for washing purposes. A blue color shows that the wash water should be acidulated for most satisfactory results.

"The desired end point of casein whey can also be determined with

sufficient accuracy for practical purposes by titrating a sample of the whey against a standard alkali solution with phenolphthalein solution as the indicator.* However, the titratable acidity value at the optimum end point varies with the different methods of making casein; for example, it is greater in whey of lactic casein than in whey of grain-curd hydrochloric casein, of the same pH value. This apparent difference in the results of the titration and color methods can be understood when it is stated that the titratable acidity, which is expressed in percentage of acid, is a measure of the quantity of acid and not a measure of acid intensity. For ordinary commercial purposes, however, when the conditions are uniform from day to day, the titratable acidity of the whey will be found a satisfactory index of attainment of the desired end point. However, it is not as quick or convenient a method as the use of bromocresol green paper.

"Experienced makers are also able, by a physical examination of the curd, to gage rather closely whether the proper acid intensity has been attained. Usually when the curd is at or near the optimum end point it will have a characteristic 'feel,' and will not mat when squeezed together in the hand. Another, though less reliable indication of proper precipitation is the presence of a clear, greenish-colored whey. The reaction of samples of the curd to wash waters of different temperatures is also an indication of whether sufficient acid intensity has been attained. These factors, together with proper testing, should all be considered in controlling the acidity of the whey."

Draining and Washing

After precipitation of the curd, the whey is drained off, either continuously or at one time, depending on whether a continuous or batch method is used. It is important to remove the whey as quickly as possible after securing the desired condition of the curd. Curd left too long in contact with the whey is more difficult to wash thoroughly and yields casein of low solubility. Draining the whey, as well as the wash water, through a drain bag prevents unnecessary loss of curd particles.

Thorough washing of the curd is very important from the standpoint of removing as much as possible of the acid, milk sugar, soluble salts, and whey constituents. Well washed curd insures casein of good color, while lack of proper washing may easily give off-colored casein. Any lactose[28] or amino acids from decomposition of moist casein[19, 26] reacts with casein during drying to produce a brown-colored product. Too much fat in the curd likewise develops poor color during drying.[42] In washing curd in vats with water, the mixture must be stirred well. The

* When the acidity is determined by titration it is customary to express the result arbitrarily in terms of percentage of lactic acid in the whey even when some other acid is used to precipitate the casein.

large vats with mechanical agitation help materially in washing the curd handled in them.

All curd should be well washed at least twice, and more often in some cases. Precipitation at higher temperatures with sulfuric or hydrochloric acid yields large clots of curd not favorable to efficient washing. Even under such conditions washing, if done as recommended, materially improves the product.

De-watering the Curd

The washed curd contains a high proportion of water which should be reduced to from 55 to 65 per cent before drying, both to reduce contamination and to facilitate drying.

The method of de-watering depends upon the type of curd; with some types de-watering may be accomplished by draining for a few hours, with others hand or continuous power presses may be used. The continuous power presses are particularly adapted to the continuous methods of precipitation but may also be used to advantage with the intermittent methods.

In using a hand press a bottomless form 24 inches square and 8 inches deep, made of surfaced boards, is placed at the bottom of the press rack and lined with press cloths. After placing the proper quantity of wet curd in the form, the cloths are turned over from both sides so as to hold the curd securely when pressure is applied, special care being taken with the corners where trouble is most likely to occur. The form is then removed and a dividing board placed on top of the bundle of wet curd; the form is then placed on top of the dividing board and the process continued until all the curd has been provided for. The amount of curd that may be satisfactorily pressed in one cake depends upon its condition. A quantity giving a well pressed cake 2½ to 4 inches thick is ordinarily most satisfactory.

When all the curd has been placed in the cloths, pressure is applied by means of the press screw. Repeated tightening of the press screw is necessary because expulsion of water from the curd quickly releases the pressure. When pressure is first applied care should be taken to see that the cakes press evenly. Later the press screw need be tightened only occasionally. Use of heavy railway car springs in hand presses as shown in Figure 8 maintains the pressure with less attention. If heavy pressure is applied too quickly the curd cakes may start pressing unevenly and have to be removed and rearranged. Hand-pressed curd contains from 50 to 65 per cent of water, depending upon the type of curd and the degree of pressing.

Grinding Curd

The curd should be ground and prepared for drying promptly when in proper condition; if it is allowed to stand too long decomposition sets in.

When the curd has been sufficiently de-watered, either by pressing or draining, grinding gives a fine, mealy curd that spreads well and dries readily. If the curd is too wet when ground, it cannot be spread satisfactorily but gathers in lumps whose surfaces dry to a hard, impervious, bone-like condition while the centers remain moist and dry very slowly, a condition known as case hardening. Properly pressed and well ground curd dries more rapidly and with less steam, yields casein of better appearance, and increases the capacity of the drier.

Drying

Drying should begin promptly after grinding and should be completed without interruption to keep the curd from molding and deteriorating. In small plants it is common practice to shovel the ground curd on a tray and spread it out by hand but better results are obtained with a shaker screen like those used for screening sand and gravel. A box arrangement with a hopper to receive the ground curd and provision for pushing the empty trays under the screen can be made easily and aids greatly in securing a uniform spread of curd over the trays in minimum time. A still better arrangement, of course, is a curd mill made with provision for spreading the curd on the trays as it comes through the mill, as shown in Figure 11. If the curd is not spread uniformly the time of drying is increased to that required by the thickest parts. Too thick a layer of curd not only retards drying but tends to affect color and quality adversely.

Control of the temperature and humidity of the air is important in any type of drier. Recirculation of a portion of the heated air through the drier, especially during initial drying, prevents case hardening of the curd particles, facilitates the balance of the drying process, and effects a saving in the heat units required. The data of Table 9 show typical distribution of temperature and relative humidity through a tunnel drier during operation.

The temperature and time of drying depend on: (1) The physical condition, moisture content and fineness of grinding of the curd; (2) the type of drier; (3) the volume and temperature of the air circulated through the drier; and (4) the uniformity and thickness of the layer of curd spread on the trays.

With poorly washed curd of a type not easily washed thoroughly, a drying temperature of 125 to 135°F. should not be exceeded; such temperatures should dry the curd thoroughly in six to seven hours. Higher temperatures may cause objectionable discoloration. A reddish-brown product commonly called burned casein results from drying poorly washed curd at too high a temperature.[19, 28] With well washed curd, temperatures as high as 155 to 165°F. can be used and the drying completed in approximately four hours. Drying at higher temperatures

TABLE 9.—Temperature and Humidity of Air in a Tunnel Drier during the Drying of Casein*

Time of observation	Condition of air: At tunnel inlet — Temperature (°F.)	Relative Humidity (%)	At tunnel outlet — Temperature (°F.)	Relative Humidity (%)
Start of run	127	0.5	64	66
After 1 hour's drying	120	9.0	100	33
After 2 hours' drying	124	8.0	105	26
After 4 hours' drying	128	11.0	114	17
After 6 hours' drying	120	15.0	120	15
After 7 hours' drying	124	12.0	124	12

* (Unpublished data from tests made by J. T. Bowen, Bureau of Dairying, U. S. Department of Agriculture, in a commercial tunnel drier. The average air velocity in the tunnel was 1,558 feet per minute, the average volume of air passed through the tunnel 2,454 cubic feet per minute.)

impairs the solubility of casein in borax, rendering it unacceptable for paper coating, and makes its solutions in alkalies more viscous.[45]

To get a good product that will keep well it is important to dry the casein thoroughly before it is removed from the drier. It is easy to tell when a properly ground curd is dry, but a coarsely ground or matted one must be examined carefully. The large particles must be broken to see whether the inside is sufficiently dry. It is also important to examine places where the curd was spread on the trays in the thickest layers.

Properly dried casein comes from the drier in a fine, granular condition, while that dried at too high a temperature, especially at the beginning, has a tendency to dry in sheets and is harder to grind.

Using the continuous drier with a higher drying temperature, drying can be completed in approximately forty minutes, giving casein of improved color and appearance.

Casein dried in tunnels is collected by jarring the trays over a bin or hopper connected with an elevating conveyor for delivery to the grinder or into sacks. With smaller quantities the dried casein may be dumped on a clean floor and then shoveled into sacks.

Grinding, Packing and Storing Casein

Grinding dried casein gives it a more uniform grain and appearance and a lighter color and allows a greater weight to be packed in a bag of given size. Some dealers, however, prefer to buy the casein unground because it gives them a better idea of the manufacturing conditions and permits them to grind it to suit their requirements.

Several types of grinder are used for grinding casein. An ordinary burr grinder, though serviceable, is not as satisfactory for heavy duty as some of the other types. In grinding casein a magnet of sufficient strength to collect nails, screws, or other pieces of metal should be

placed in the feed pipe to keep them from injuring the grinder. Pieces of metal coming through the grinder may also ruin the screening cloth when a screen is used.

If the ground casein is to be sold on the basis of a certain particle size it should be screened and the coarse particles returned to the grinder.

The finished casein, either ground or unground, should be packed in suitable burlap sacks, using liners of strong paper or closely woven cloth with the finely ground product. A paper bag of three or more plies conforming to all shipping requirements is available and is used by some manufacturers for the shipment of casein.

When held for any length of time the casein should be stored in a dry, clean place with as little change in temperature as is possible. It is well to bear in mind that insects will work in ground casein but rarely are found in the unground product (see Chapter 6).

Yield of Casein

The yield of casein depends upon the composition of the skim milk and the method and manner of manufacturing. It varies between 2.7 and 3.5 per cent but with skim milk of average composition the yield of well dried casein containing not more than 8 per cent moisture usually ranges from 2.7 to 3.1 per cent. The condition of the curd and care in handling affect the yield materially. There is of necessity more chance for losses and reduced yield with the methods giving a finer curd and requiring more handling during washing and draining, but any decrease in yield should be offset by the increase in quality. When properly followed any one of the methods described gives a normal yield for the composition of the skim milk used.

Some fine curd may result in the acid-pressed and cooked curd methods from the use of too much acid or an improper method of adding it, as well as from lack of control of temperature.[31] The yield of cooked curd casein may be materially reduced by the addition of too much acid, which produces much fine material that is easily lost with the equipment ordinarily used in making this type of product.

Lactic Acid Casein

Vat Method

This method, formerly quite commonly used, is now desirable only where smaller quantities of skim milk are handled. Fresh skim milk from the separators is set in vats at a temperature ranging from 90 to 110°F., depending upon the time in which precipitation is to be accomplished. The lower temperatures are said to produce lower ash content in the product.[25] The addition of 5 to 10 per cent of sour whey from a previous lot, together with a higher temperature, materially shortens the time required. Just before the skim milk coagulates, at which time

the acidity is from 0.45 to 0.6 per cent and the pH is approximately 4.6,[46] it is heated by direct steam while being stirred constantly with a wooden rake until there is a clear separation of the curd and whey. A temperature between 110 and 125°F. should give the desired results, the curd collecting at the bottom of the vat in a more or less coherent mass, leaving the whey clear and free from small particles of curd. The lower temperatures produce a curd that can be washed more thoroughly, yielding a product of lower ash content, but if the temperature is too low there is loss of very fine particles of curd.[5, 25]

The clear whey is drained through a drain bag tied on the gate valve to catch curd particles that might otherwise be lost. Sufficient whey is saved and soured for the next day's use. The curd is washed at least twice by covering with cold water and stirring well each time to break it into smaller particles. After the wash water is drained, the curd is ready for pressing.

Too high an acidity of the skim milk at the time of heating gives a finer curd, which is harder to handle without using a drain rack, whereas insufficient acidity prevents a clear and efficient separation of the curd. Experience soon teaches the operator the proper acidity and conditions for best results. With a good, active whey starter in the maximum quantity mentioned, skim milk set at the higher temperature should develop sufficient acid within two to four hours to produce a clear separation of the curd when heated. At the lower temperature four to six hours may be required.[46] Since heat is given off during the souring of milk there may be a rise in temperature of 2 or 3°F. during the process.[20]

When the skim milk is allowed to stand over night, as is quite often done, the temperature at which it is held and the quantity of whey starter used should be regulated to prevent the skim milk from reaching the stage of coagulation too long before it is to be heated. If the skim milk coagulates and then stands for some time, a higher temperature of heating must be used to get the curd in satisfactory condition for handling without a drain rack, but it should not exceed 135 to 140°F. in any case. Stirring less vigorously when heating highly acid skim milk lessens the disintegration of the curd into fine particles.

When skim milk sours without the addition of a starter there is often an increase in pH at the beginning, followed by decrease to pH 4.6 where coagulation occurs, but the titratable acidity increases continuously from the beginning of the process.[21]

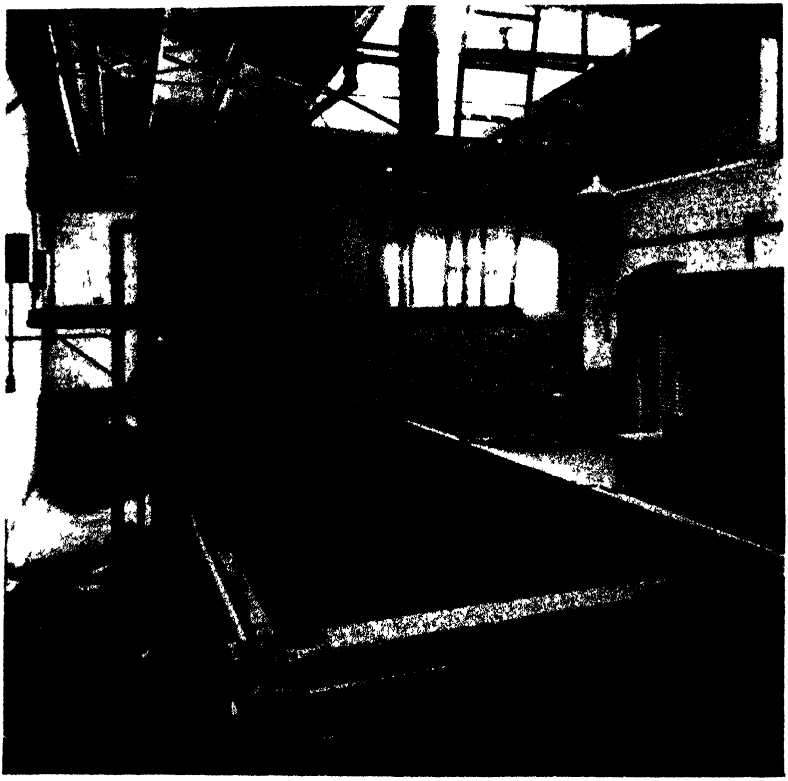
According to Trimble and Bell[40] cooked curd lactic casein may be made as follows:

"After the curd has been precipitated by direct steam at a low acidity (0.45 to 0.50 per cent acid) and the whey drained off, the curd is covered with warm water, and the water and curd heated to 160 to 180°F. This

high temperature causes the curd to gather in a rubbery, plastic mass. The curd is then packed in barrels where it settles into an almost air-tight solid mass, which, upon cooling, becomes very tough and hard. The filled barrels are then usually shipped to a central drying plant where the curd is ground and dried. When a green curd, either pressed or cooked, is shipped to a central drying plant it is usually paid for on the basis of the dried casein obtained. Casein made from cooked curd has a high ash and free acid content, is difficult to dissolve in the usual casein solvents, makes a heavy-bodied solution, and may be very unsatisfactory, depending on the purpose and method of utilization."

Vat Method with Agitator

A better and quicker method of making lactic acid casein by the batch method makes use of a large Jumbo, round bottom, wooden vat, holding



Courtesy of Universal By-Products Co.

Figure 16.—A large casein vat with mechanical agitator in the background; below it, in the foreground, a drain vat with false bottom of perforated iron.

15,000 to 20,000 pounds of skim milk and provided with a means of agitation (Figure 16). Highly sour whey from a previous batch is added to the skim milk in the vat at 95 to 110°F. while running the agitator. The curd precipitates quickly and is easy to wash. After draining the whey, water is added and the mixture agitated for washing, after which the water is drained and the washing repeated once or twice. This curd can either be pressed with a hand or continuous press, or can be de-watered sufficiently for grinding and drying by draining in a vat provided with a false slotted or perforated iron bottom, as shown in Figure 16.

Universal Continuous Lactic Acid Precipitation Method

A method of continuous lactic acid precipitation used to a considerable extent on the Pacific Coast, known as the Universal Process, is an improvement made by J. R. Spellacy of the Universal By-Products Company on an earlier continuous precipitation process. With this method highly acid whey is used as the precipitant. The whey is soured by holding it at temperatures favorable to the development of a high content of acid and should contain one per cent or more at the time used.

The equipment required for handling 50 to 100,000 pounds of skim milk includes three 1,000 gallon tanks, one for skim milk and two for whey, a round-bottom vat with agitators holding approximately 15,000 pounds of skim milk, drain vat and necessary piping. Figure 17 shows the flow sheet and the equipment used in this process.

Precipitation is effected by mixing heated skim milk and high-acid whey by means of mixing tees in the lines leading from the storage tanks. For average conditions a temperature near 110°F. is required for proper precipitation and should be thermostatically controlled. The heated mixture of skim milk and highly acid whey is run into the large vat by flowing over a riffle box, Figure 18, which enables the operator to observe the precipitation more closely. The riffle box is a plain wooden box with one end closed, 6 to 8 feet long, 12 inches wide, and 8 inches deep, with a bottom of corrugated iron. Although the riffle box is not essential to the process its use is recommended.

The volume of skim milk and highly acid whey is adjusted to give a clear precipitation, at which the acidity of the mixture averages 0.35 to 0.40 per cent. Enough whey should be used to give a complete separation; a trifle too much does no harm.

The curd settles to the bottom of the vat and as the whey rises it is drained off through an overflow pipe on the front of the vat and pumped to storage tanks or otherwise disposed of. When one whey storage

tank is empty it should be refilled with fresh whey and held at warm temperature for later use.

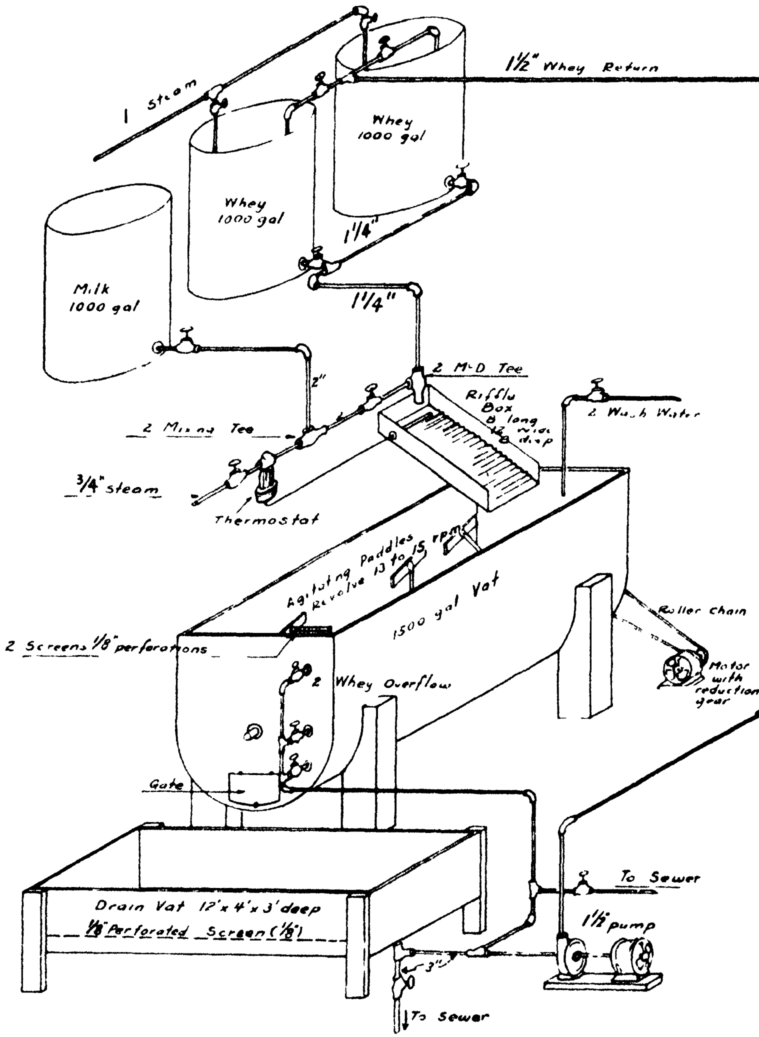


Figure 17.—Flow chart of the Universal continuous precipitation method.

When the vat is not more than half full of curd, the flow of whey and skim milk is shut off and the whey drained down to a point where there is just enough to cover the curd. Unheated, highly acid whey is then

added while running the agitator to bring the acidity of the whey in the vat to one per cent. The curd takes on a yellowish cast at this acidity and it is important to make sure that the acid has thoroughly penetrated the curd, which can be determined by breaking particles of it and noting the color of the interior. If the interior does not have a yellowish cast, more highly acid whey should be added or additional agitation given. If the curd is not brought to the proper acidity at this stage the quality is impaired and the casein has a tendency to dry in sheets and stick to the trays.



Courtesy of Universal By-Products Co.

Figure 18.—A casein vat showing agitator and riffle box as recommended for the Universal continuous precipitation method

Next the whey is drained, the curd covered with water, and washed by running the agitators for a few minutes. The washing is repeated and when the water is drained the curd is washed into a drain vat with a false bottom or a drain rack of perforated iron. The curd can be pressed immediately or can be ground without pressing if allowed to drain for two or three hours. When manufacturing conditions are

unfavorable, a longer draining time may be required before grinding without pressing.

When handling smaller quantities of skim milk by this method smaller storage tanks may suffice. Rectangular tanks for receiving the precipitated curd, like those of the standard batch method, and small racks for draining may be used. The method of operation is essentially the same.

Sulfuric Acid Casein

Pressed Curd Method

The fresh skim milk is heated to 120°F., never above 125°F., in the rectangular, flat-bottom casein vats. Commercial sulfuric acid is diluted with at least four to six times its own volume of water by adding the acid carefully to the water. The diluted acid is added slowly while stirring the skim milk constantly until a good separation of the curd is obtained, as indicated by the clearness of the whey. This requires but a few minutes provided sufficient acid is used. If the curd does not separate well, leaving a clear whey, more acid is gradually added, but no more than is required for good separation of the curd. The proportion of acid consumed depends upon the acidity and temperature of the skim milk. The curd is pushed back from the gate valve, and after draining, the whey is covered with cold water for washing. This curd is difficult to wash effectively because it is in large clots; the clots should be well broken up and washed at least two or three times.

After draining the wash water, the curd is ready for pressing. This curd should be pressed exceptionally well to remove as much of the free acid and impurities as possible.

Cooked Curd Method

This method eliminates pressing the curd and requires practically no equipment other than the precipitating vats. It may be used when a plant wishes to sell wet curd, and is located where it can be shipped to a central drying plant.

Cooked curd caseins are not of high quality. They are high in content of ash and acid, difficult to dissolve without extra solvents and high in viscosity and are therefore becoming increasingly difficult to market.

The process of precipitating is the same as that used for pressed curd; the skim milk is heated to 120°F. and diluted sulfuric acid added at the rate required to cause proper separation of the curd and whey. After draining the whey, the curd is broken up in a vat, covered with water and the mixture heated to 170 to 175°F. by direct steam. At that temperature all the curd should collect in a semifluid, tough mass. The water is drained and, after kneading the soft curd to remove as

much moisture as possible, it is placed in barrels where it settles into an almost air-tight mass, which, upon cooling, becomes very tough and impervious and will keep for several days, even in hot weather. Fresh cooked curd can be put on top of the hardened curd in a barrel partially filled from a previous batch. Barrels filled with cooked curd and covered with burlap can be shipped to a central drying plant. Cooked curd is hard to grind and requires an especially strong mill (Figure 12).

No more acid than is required to give a clear separation should be used because an excess seriously interferes with the proper working of the cooked curd method. When the proper percentage of acid is used in precipitating the curd the clear whey shows a titratable acidity between 0.30 and 0.32 per cent, using phenolphthalein as indicator. If it shows more than 0.34 per cent acidity the curd when cooked in the water does not collect in the characteristic plastic mass but breaks up into fine particles, making it difficult to handle.

When the cooked curd is to be dried at the plant where it is made, time and expense may be saved by handling it in some manner other than packing in barrels. If sufficient clean cement floor space is available the hot plastic curd may be deposited in batches and allowed to spread out on the floor in flat chunks that can be handled sooner and more easily. The curd may also be allowed to remain in the bottom of the vat to drain until ready for grinding if the vat is not needed for additional batches.

Hydrochloric Acid Casein

Grain Curd Casein

The grain curd hydrochloric acid method was developed by the Bureau of Dairy Industry[11, 38, 40, 47] for the purpose of producing a uniform casein of high quality.

Quoting from Trimble and Bell:[40] "Fresh skim milk is heated to 93 to 96°F., depending upon its acidity. For best results skim milk that has an acidity of not more than 0.15 to 0.16 per cent lactic acid should be heated to 96° and skim milk with an acidity of 0.20 to 0.21 per cent should preferably be heated to 93 or 94°. Close control of the precipitating temperature within these limits is essential to obtain a firm, granular curd. A temperature above 96° when the ordinary vat method is used, tends to make a coarse, lumpy curd, and a temperature below 93° gives a soft, fine curd, both of which are detrimental to the product. After the skim milk has been thoroughly stirred and heated the foam should be scraped off with a suitable board or level before the acid is added, as foam tends to make a floating curd.

"There are two important factors to consider in adding the acid to the heated skim milk, namely, the dilution and the method of adding. The hydrochloric acid should be diluted by mixing 8 to 10 parts of water with 1 part of acid. The dilute acid should be added slowly in a spray or fine stream to the skim milk, which should be stirred gently and constantly to insure even distribution of the acid and to prevent localized precipitation. If the mixture of skim milk and acid is not stirred, the curd will form uneven masses. Very fast stirring or a temperature below 93°F. gives too fine a curd, which tends to float, is hard to drain and wash properly, and causes mechanical losses in processing and foaming in the finished product. Too slow stirring or a temperature above 96°F. gives a coarse, lumpy curd which is difficult to wash effectively. When the curd begins to 'break' or settle in the vat it is important that stirring be continued until the whey is clear, or practically all of the curd has been precipitated. The curd, however, is still soft and mushy, and more of the dilute acid should be added slowly until the whey has an acid content corresponding approximately to pH 4.1 in a color test with bromocresol green paper, or 0.50 per cent acid by titration. The total amount of concentrated acid required is affected slightly by the percentage of lactic acid in the skim milk; the casein content of the milk, which varies with the breed of cows and the season of the year and the feed given the cows, the precipitating temperature, and various other factors. Approximately 2.4 to 2.6 quarts of concentrated acid with a specific gravity of 1.15 per 1,000 pounds of fresh skim milk will usually be needed. The correct end point, expressed in titratable acidity, may range from 0.47 to 0.53 per cent acid, but it will normally be 0.50 per cent.

"When the correct end point is reached, the curd will have a characteristic 'feel' and appearance. The curd is firm, porous, resilient, and will not mat when squeezed together in the hand. With a little practice the maker, by examining the curd, can gage this point rather accurately and consequently determine when the right amount of acid has been added. Insufficient acid gives a milky, soft curd that breaks up easily in washing and reduces the yield. An excess of acid causes a gelatinous sticky layer to form over the curd particles, which retards thorough washing and injures the texture. Some of the whey may be drained before the correct end point is reached. Slightly less acid will be required when this is done. The correct end point should be attained as soon as possible and the curd drained and washed quickly for best results.

"Effective washing of the curd is very important in making grain curd casein as in making other types of casein. The curd should be

washed at least twice and preferably three times. The best temperature for all except the last wash water is 94°F. About as much water is added to the curd as there was whey in the vat, the curd is stirred thoroughly, and the water drained off. This operation is repeated once or twice. The use of water that is much cooler than 94° in the first washing tends to soften the curd, causes it to stick to the press cloths, and retards drainage in the press. The use of water that is too hot tends to cook the curd and to make the finished casein more insoluble. Either extreme should be avoided. The last wash water, if desired, may be cooler; water with a temperature as low as 60° is often used in the final washing. It is advisable to acidulate the first wash water by adding hydrochloric acid at the rate of about 100 cubic centimeters of acid to 1,000 pounds of water.

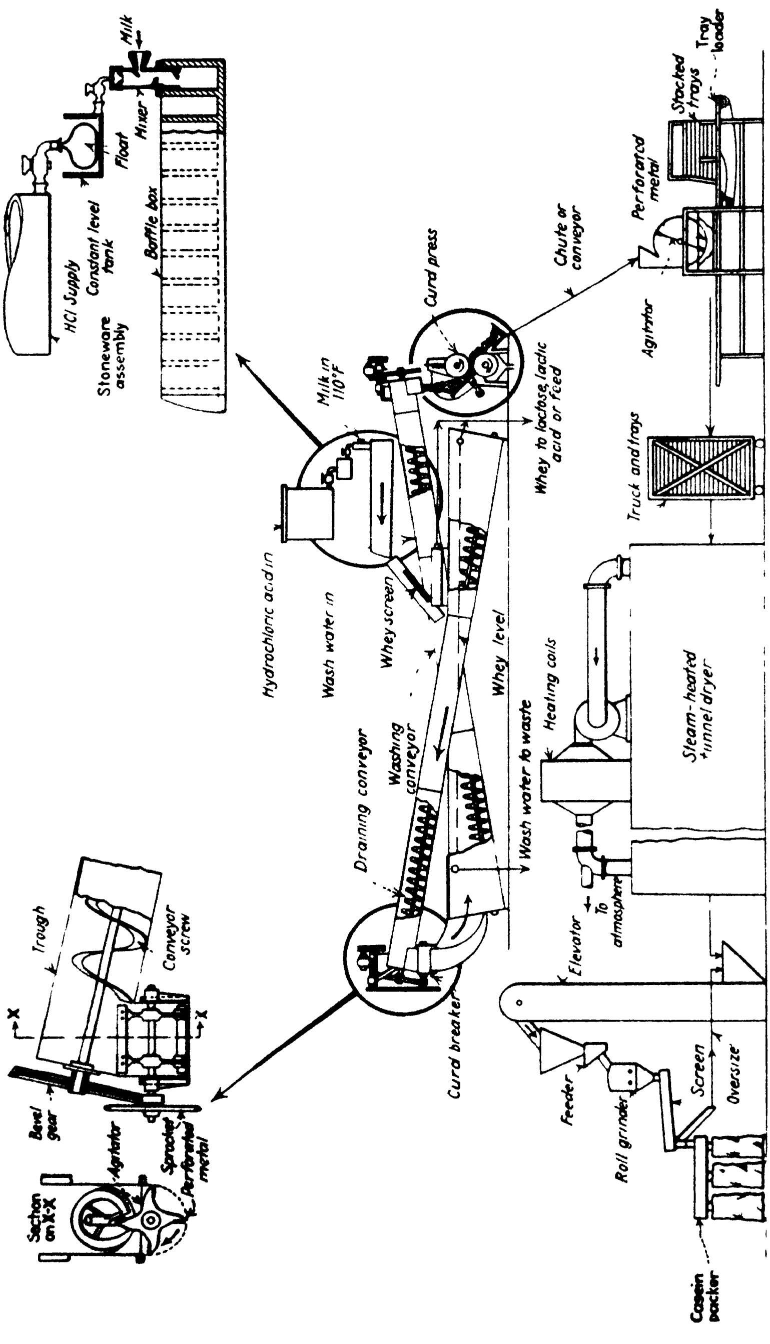
"The curd is now ready to be pressed and is handled throughout the rest of the process according to the usual methods."

Sheffield Continuous Process

The Sheffield continuous process was developed to produce casein of uniformly high quality with maximum efficiency in plant operation.[8, 10, 33, 35] Continuous precipitation, washing, and pressing is effected in a machine shown in Figure 20. The process is shown digrammatically in Figure 19.

Quoting from Chemical and Metallurgical Engineering:[10] "Skim milk under thermostatic control is heated continuously to 110°F. in a concentric pipe heat exchanger, using hot water as the heating medium. Hydrochloric acid of 20° Baumé concentration is withdrawn from a rubber-lined storage tank and diluted with four parts of water and pumped to a 125-gallon chemical stoneware vessel. From this it flows at the required rate, as determined periodically by titration, through a float-controlled constant level tank and stoneware cock into a small stoneware cylinder containing baffles and serving as a mixer. Here it meets the thin stream of warm milk, mixes with it instantly and precipitates most of the casein curd. To give time for the completion of the reaction, the curd, whey and unreacted milk and acid flow through a stoneware baffle box, discharging over a screen into the draining conveyor. A considerable part of the whey passes through the screen and is withdrawn, together with the whey from the draining conveyor, to further processing for the production of feed or the recovery of lactose or lactic acid.

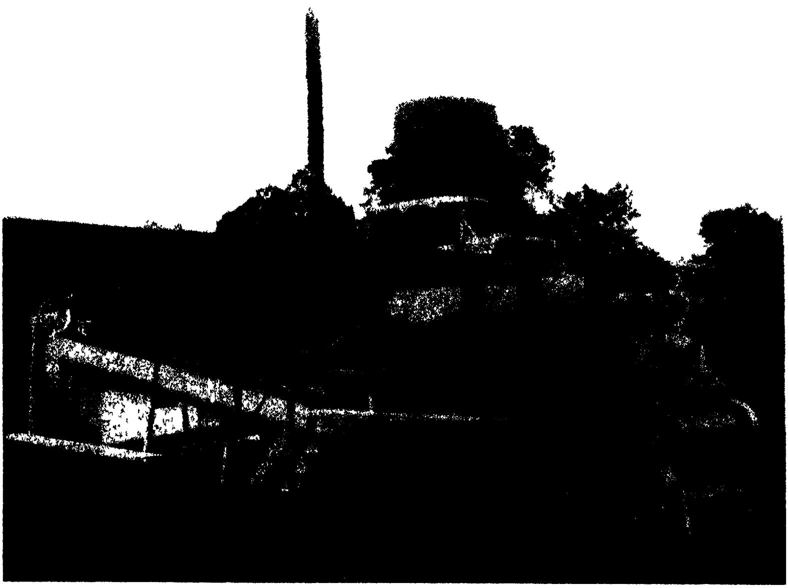
"The draining and washing conveyors are of similar construction, consisting of steel-ribbon type screw flights inclined at an angle of 9° to the horizontal. The lowest section of trough for each conveyor is



Courtesy of Chemical and Metallurgical Engineering

Figure 19.—Flow chart for the Sheffield continuous process.

designed so that its upper edge parallels the liquor level. Whey and curd drop into the first conveyor at a point slightly below its center. The whey gravitates towards the discharge, while the curd is moved upward by the flights, being rolled over constantly in its travel and broken down to a certain extent. By the time it reaches the discharge to the curd breaker at the uppermost point of the draining conveyor (extreme left in Figure 20), it has lost as much whey as can be removed by draining.



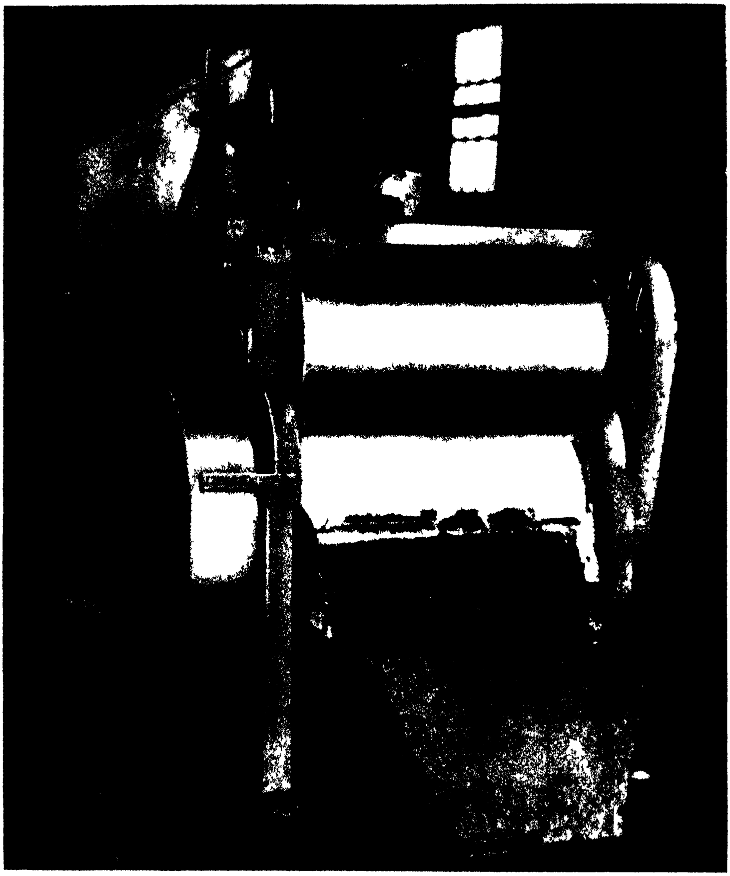
Courtesy of Chemical and Metallurgical Engineering

Figure 20.—The precipitating, washing, and pressing machine used in the Sheffield continuous process.

"Dropping into the curd breaker, the curd is further broken down and pushed through a perforated plate by a rotating agitator. This prepares the material for the thorough counter-current washing which is the next step. Curd drops down a chute and is picked up by the washing conveyor which moves it slowly upward through a stream of cold wash water. By the time the particles have been carried to the upper end of the wash conveyor the ash content has been reduced to about 1 per cent (based on burning the casein in a muffle furnace) and the free acidity to a fraction of a per cent."

The washed curd is then passed directly into a two-roll continuous power press (extreme right in Figure 20 and Figure 21), the upper

chromium-plated solid roll through spring pressure forcing the curd against the lower perforated brass roll. Variations in the speed of the rolls allow synchronizing with the delivery of curd from the conveyor. An oscillating plate operated by an eccentric serves a double purpose,

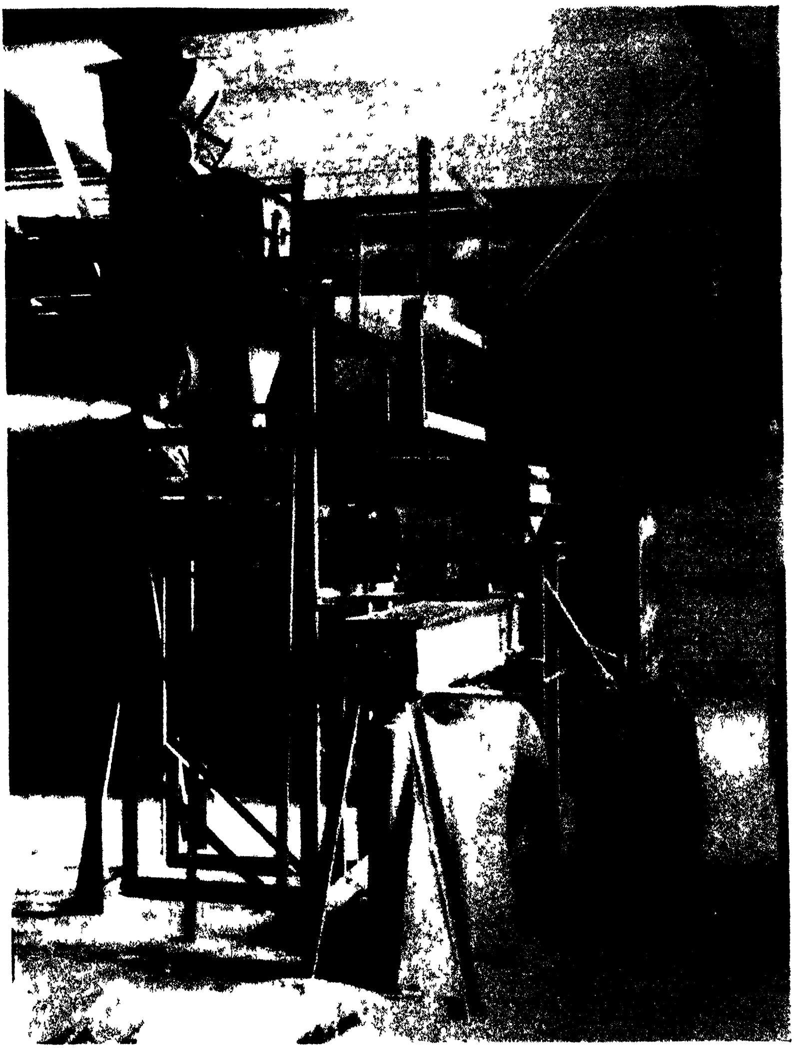


Courtesy of Chemical and Metallurgical Engineering

Figure 21.—The two-roll continuous curd press in the Sheffield process

acting both as one side of a hopper and as a means of forcing the curd between the rolls. The pressed curd is scraped from the lower roll with a wooden knife and is in fine condition for drying either in a continuous or tunnel drier. The low-acid curd produced by this process withstands a higher drying temperature without discoloration, and the higher temperature reduces the drying time. Claims are made for this casein that

it may be stored for a much longer period than the higher-acid casein without discoloration or loss of adhesive qualities.



Courtesy of Chemical and Metallurgical Engineering

Figure 22.—Grinding, sifting, and packing dry casein

Spellacy Continuous Process

The Spellacy continuous process[36] likewise requires a special apparatus for producing casein of uniformly high quality with maximum plant

efficiency. Skim milk or buttermilk is heated by a jet of steam in a thermostatically controlled T-tube mixer and acid is run in at a second T-tube mixer of acid-proof material. The acid may be dilute hydrochloric acid, sulfuric acid, ethyl sulfuric acid, or a mixture of one of these acids and sour whey. The coagulated mixture then runs into an inclined riffle box. Within certain limits the slope of the riffle box controls the size of the particles of curd, a steeper slope favoring formation of smaller particles. From the riffle box the mixture flows to a vibrating screen which retains the curd and allows the whey to pass through. The screen is inclined sufficiently for the vibration to work the casein downward as it drains. The curd discharges on an inclined, stationary apron, whence it passes through wringing rolls for de-watering and thence to a curd grinder. From the grinder the curd passes down a second inclined, vibrating screen where it is sprayed with wash water. The washed curd may then be pressed and dried by methods already described.

The Spellacy process has the advantage of separating the curd from the whey very promptly after precipitation. It yields a product of very low ash content and high quality.

Pressed Curd Hydrochloric Acid Casein

The fresh skim milk is heated to 115° to 120°F., never exceeding 120° because the higher temperature gives a much less desirable casein. Commercial hydrochloric acid diluted with two to four parts of water, by adding the acid to the water, is gradually added to the skim milk while stirring continuously until the curd precipitates properly. Approximately 2½ pints of acid (diluted by pouring into 2 to 4 parts of water) is required for every 1,000 pounds of skim milk. Care should be taken not to add acid beyond the point giving proper separation since an excess gives a soft curd, which is difficult to handle. Adding the diluted acid gradually and stirring well after each addition helps to insure against using too much.

The hydrochloric acid curd precipitated by this method is inclined to be soft and may give trouble sticking to the press cloths. To overcome this difficulty, the curd from which the whey has been drained is covered with luke-warm water, and a few ounces of sulfuric acid, well diluted with water, is added for each 1,000 pounds of skim milk, to make the curd firmer and easier to press.

Hydrochloric Acid-cooked Curd Casein

This method eliminates the pressing of the curd and requires little equipment other than the precipitating vats. It may be used by creameries desirous of selling wet curd when they are so located that the curd can be shipped to a central drying plant. The process of precipitating the curd is the same as that for hydrochloric acid-pressed curd, but the

treatment with sulfuric acid water is unnecessary. The curd is covered with cold water and heated with direct steam to 175 to 185°F. which fuses the curd into a semifluid, plastic, tough mass, providing too much acid has not been used. The same factors affecting the proper working of the method mentioned under sulfuric acid-cooked curd apply to this method. After draining the water the plastic curd is kneaded to remove as much moisture as possible, and handled as described under sulfuric acid-cooked curd casein.

Rennet Casein

Rennet casein, to possess the properties desired by the trade, must be made from absolutely fresh skim milk, low in content of fat, before it has had a chance to develop any appreciable acidity. Skim milk that has become acid yields rennet casein too low in content of ash[5, 34] although Brigando[5] reports that a product of normal ash content can be obtained by neutralizing the acid before precipitating with rennet. Rennet casein is used extensively in the manufacture of plastics, for which purpose it must be made carefully to meet the strict requirements of that industry. Good equipment kept scrupulously clean is required. The users of rennet casein usually demand that the product meet certain standards, the most important of which are that the ash content shall be not less than 7.5 per cent, fat not more than 1.0 per cent, and that it have a good bright color and be free from all particles of dirt or foreign matter of any kind.

Precipitating the Curd

The perfectly fresh skim milk is set at 96°F. with sufficient rennet, properly diluted with water, to bring about the required coagulation in 15 to 20 minutes. The quantity of rennet required varies according to its strength and the condition of the skim milk. With perfectly fresh skim milk the quantity ranges from 70 to 100 cubic centimeters for each 1,000 pounds of skim milk, while for skim milk in which a slight development of acidity has taken place the quantity ranges from 40 to 60 cubic centimeters. The time for cutting or breaking the curd and heating is important and must be adjusted to meet conditions. A good rule to follow is to note the time from the addition of rennet to first signs of coagulation, and add approximately one-tenth, or whatever fraction experience shows to give the desired results, to this period, as the time for starting to cut or break up the coagulated curd. Some directions for making rennet casein recommend continual stirring of the skim milk during coagulation, or heating to a higher temperature than that mentioned. Heating the mixture, either by a steam jacketed vat or by direct steam, properly admitted by means of an arrangement to prevent the curd burning on the pipes, is commenced shortly after

starting to cut or break up the curd, and continued until the temperature reaches 150°F. After allowing the curd to settle for ten minutes the clear whey is drained as quickly as possible through gate valves on the end of the vat. A series of gate valves at different levels helps in draining both the whey and the wash water from the top down while the curd is settling. The curd is immediately covered with water at about 75 to 80°F. and the mixture stirred for several minutes, taking special care to loosen all curd from the bottom of the vat. After standing for several minutes the water is run off into the cloth-lined drain rack, and the process of washing repeated once or twice, keeping the temperature of the curd between 80 and 90°F., because lower temperature of the curd has a tendency to give a harder product when dried. The curd remaining in the vat is run onto the drain rack with the last wash water and allowed to drain.

Pressing and Drying

Extra care must be exercised in the pressing and drying of rennet curd because it gives up moisture quite readily. It should be pressed heavily for an hour and then ground and spread thinly on the drying trays to facilitate quick drying at a temperature of 100 to 115°F. A higher temperature or slow drying gives a dark, undesirable color.

Close attention to details is required to make a good quality of rennet casein, but by handling the skim milk when it is very fresh and taking the proper precautions no real difficulty should be encountered in obtaining a very satisfactory product.

Casein from Buttermilk

Casein may be made from buttermilk[4, 12] but it is rarely done in the United States because the product varies widely in properties, depending largely upon the composition of the buttermilk. In particular the fat content of buttermilk casein is usually much higher than that of good casein from skim milk, and for this reason the color developed on drying may be unsatisfactory.[41] The viscosity of solutions made from buttermilk casein is generally low.

Edible and Pharmaceutical Casein

There seems to be little information available about the manufacture of edible and pharmaceutical casein. Needless to say casein for this purpose should be made in a sanitary manner with unsparing attention to cleanliness and purity.[15]

Edible casein may be made by the rennet method, which may be particularly desirable because of the high content of calcium in the product. Other suitable methods are natural souring and the Van Slyke and Baker method described in Chapter 1. The curd should be precipitated

and handled in a manner that gives conditions most favorable for washing out the lactic or acetic and hydrochloric acids. Repeated washing of the curd, of course, is essential. The ordinary hydrochloric acid methods are said to leave in the product impurities detrimental to health.

Practices in Other Countries

The methods of making casein in other countries apparently are very similar to the intermittent methods used in the United States. The list of references at the end of this chapter includes descriptions of practices in Argentina,[4, 6] France,[1, 2, 5, 14, 15, 27, 43] England,[7, 13, 37] India,[29] New Zealand,[24] and Germany.[20, 21, 32, 34] In Japan a method of manufacturing casein by electrodialysis is described.[18] Skim milk is dialyzed in the middle one of three chambers separated from the anode chamber by a membrane of silk and from the cathode chamber by a membrane of sail-cloth, both membranes being coated with chromated gelatin and copper ferrocyanide, the latter to hold back lactose from diffusing into the electrode chambers. The anode is carbon and the cathode brass. Salts are removed from the skim milk by electrolysis with a direct current of 1 ampere at 100 volts and the pH decreases to the isoelectric point, where the casein coagulates. The casein is filtered off and the whey in the middle chamber may be used for recovery of lactose. There is another new process of precipitating casein reported from Germany,[17] in which pectin from ripe fruits is used as the coagulating agent in place of the usual acids or of rennet, and the resulting product is said to be superior to those produced by the older methods.

"Soluble casein," which can be dissolved in water without the addition of other chemicals, is occasionally manufactured. It is usually ammonium caseinate. According to patent claims it may be manufactured by dissolving ordinary casein in ammonium hydroxide, spray drying the solution, and recovering the excess of ammonia for further use.[16]

References

1. Barthelemy, H., *Rev. prod. chim.*, 21, 227 (1918)
2. Beau, M., "La caséine," Le Lait, Paris (1933)
3. Benton, A. G., *Ind. Eng. Chem.*, 20, 15 (1928); U. S. Pat. 1,716,799
4. Bertuzzi, F. and Teitelbaum, J., *Rev. facultad quim. ind. agr., Univ. nacl. litoral*, 2, 149 (1932)
5. Brigando, J., *Lait*, 13, 657, 843, 965, 1098, 1230 (1933); "Recherches sur la caséine," Le Lait, Paris (1933)
6. Brookens, P. F., *Natl. Butter and Cheese J.*, 24 No. 23, 12 (1933)
7. Butcher, C. H., *Ind. Chemist.*, 1, 250 (1925)
8. Chappel, F. L., *Food Ind.*, 3, 102 (1931)
9. Chappel, F. L., U. S. Pat. 1,892,233 (Dec. 27, 1933)
10. Chemical and Metallurgical Engineering editorial staff, *Chem. & Met. Eng.*, 41, 645 (1934)

11. Clark, W. M., Zoller, H. F., Dahlberg, A. O., and Weimar, A. C., *J. Ind. Eng. Chem.,* **12,** 1163 (1920)
12. Dahlberg, A. O., *U. S. Dept. Agr. Bull.,* 661 (1918)
13. Davies, W. L., *Chem. Age (London),* **20,** 23, 43 (1929)
14. Déribéré, M., *Rev. gén. mat. plastiques,* **10,** 57, 99 (1934)
15. Fouassier, M., *Bull. soc. hyg. aliment.,* **16,** 218 (1928)
16. Germann, A. F. O., U. S. Pat. 1,881,180 (Oct. 4, 1923); 1,896,300 (Feb. 7, 1933)
17. Hepburn, N. H., *Am. Creamery and Poultry Produce Rev.,* June 17, 1936, quoted from U. S. Dept. of Agr. *Daily Digest,* **62,** Sec. 1, July 2, 1936; Plawenn, A. v. and Kersten, G., U. S. Pat. 1,953,734 (Apr. 3, 1934)
18. Kato, J., *J. Soc. Chem. Ind. Japan,* **35,** Suppl. binding 158 (1933)
19. Laxa, O., *Proc. 8th World's Dairy Congress,* **1928,** 810
20. Lind, C. and Jensen, M., *Deut. Molkerei-Ztg.,* **55,** 1308 (1934)
21. Mayorov, S. N., *Z. Untersuch. Lebensm.,* **69,** 301 (1935)
22. Mohr, W., Kramer, R., and Schröter, W., *Molkerei-Ztg.,* **48,** 1054 (1934)
23. Nabenhauer, F. P., *Ind. Eng. Chem.,* **22,** 54 (1930)
24. Pedersen, J., *New Zealand J. Agr.,* **19,** 347 (1919); *Expt. Sta. Record,* **43,** 15
25. Pien, J. and Herschdoerfer, S., *Lait,* **14,** 30 (1934)
26. Pien, J. and Bergier, M., *Lait,* **14,** 155 (1934)
27. Porcher, Ch., *Proc. 8th World's Dairy Congress,* **1928,** 800
28. Ramsey, R. J., Tracy, P. H., and Ruehe, H. A., *J. Dairy Sci.,* **16,** 17 (1933)
29. Rao, T. L., *Madras Agr. J.,* **21,** 388 (1933)
30. Riley, C. L., U. S. Pat. 1,878,182 (Dec. 20, 1933)
31. Sammis, J. L., *J. Ind. Eng. Chem.,* **11,** 764 (1919)
32. Scherer, R., "Casein, its preparation and technical utilization," 2nd ed., revised by H. B. Stocks, New York, D. Van Nostrand Co., Inc.
33. Sheffield, W. H., U. S. Pat. 1,716,799 (June 11, 1929)
34. Skraup, Z. H. and Krause, E., *Monatsh.,* **30,** 447 (1909)
35. Smith, R. W. Jr., *Ind. Eng. Chem.,* **26,** 819 (1934)
36. Spellacy, J. R., U. S. Pat. 2,099,379; *Natl. Paint Bull.,* **1,** No. 13, 17 (1937)
37. Stocks, H. B., *First Report Brit. Comm. on Colloid Chem. and Its Industrial Applications,* Dept. of Sci. and Ind. Research (London), 1917.
38. Thornton, R. I., Buffalo Forge Co., *personal communication*
39. Trimble, C. S. and Bell, R. W., *Creamery and Milk Plant Monthly,* **19,** No. 8, 40 (1930)
40. Trimble, C. S. and Bell, R. W., *U. S. Dept. of Agr. Circ.,* **279** (1933)
41. Van Slyke, L. L., *Proc. World's Dairy Congress,* **2,** 1145 (1923)
42. Van Slyke, L. L., and Price, W. V., "Cheese," New York, Orange Judd Publishing Co., 1927
43. Waguet, P., *Rev. prod. chim.,* **23,** 5, 62, 115 (1920)
43a. Weimar, A. C. and Taylor, J. J., *Mich. Agr. Expt. Sta., Tech. Bull.,* **82** (1927)
44. Whittier, E. O., *J. Dairy Sci.,* **14,** 26 (1931)
45. Whittier, E. O. and Gould, S. P., *Ind. Eng. Chem.,* **24,** 1081 (1932)
46. Wood, R., *Natl. Butter & Cheese J.,* **24,** No. 13, 16, 33 (1933)
47. Zoller, H. F., *J. Ind. Eng. Chem.,* **13,** 510 (1920)

Chapter 5

Testing and Analysis of Caseins

EDWIN SUTERMEISTER

Commercial caseins vary widely in important characteristics affecting their practical use. Rennet casein, with its exceedingly high content of ash, stands apart from the acid caseins and is demanded by manufacturers of plastics because the moist gel possesses a degree of plasticity particularly well adapted to the extrusion process upon which the casein plastics industry depends. For the other industries using casein, however, rennet casein is objectionable because its solutions are relatively much too viscous; the acid caseins, with low ash content and relatively low viscosity, are therefore required. In all the industries uniformity in properties from shipment to shipment is exceedingly important. Difficulty in obtaining uniformity in caseins produced in small plants by batch methods has in the past been a serious handicap to the industries using casein. Fortunately there has been great improvement in this respect during the past decade or so.

As a rule the most satisfactory method for determining the suitability of a casein for a given use is to try a sample of it in a laboratory batch of the product, following the formula to be used in practice. For the buyer the laboratory batch is often a very convenient way of testing caseins for uniformity and suitability for his purpose, but the seller is usually in no position to make similar tests. Many efforts have therefore been made to devise simple physical or chemical tests for evaluating casein in order to provide an adequate basis of agreement between buyer and seller or for incorporation in formal specifications.[4, 8, 10, 12, 17, 19, 21, 23, 24, 30, 36, 40, 43, 45] Although no system of testing has yet been developed that will fully predict the behavior of a casein in any given product made from it, a wise selection of arbitrary physical tests, together with proximate chemical analysis, can be used successfully for securing reasonably uniform purchases of whatever type of casein has been found suitable for the purpose in question.

Physical Tests

The physical tests that have been commonly used include observations of cleanliness, color, odor, fineness, solubility in a standard alkaline solution, viscosity of a standard solution, and "strength."

Cleanliness

The best way to judge the cleanliness of casein is by examination under a microscope at low magnification, or by the use of a good hand lens. In the absence of such facilities it may be subjected to careful visual inspection of the material as received. A third method is to make a moderately dilute solution, say 25 grams of casein in 500 cubic centimeters of water containing 2 grams of sodium hydroxide, allow it to stand for a short time, and note the dirt that settles out or floats on the surface. When casein is examined in this way an idea can frequently be gained of the nature of the dirt and sometimes of its source.

Absence of dirt, or at least the presence of a minimum amount, is important in most of the industries in which casein is used. The most notable exception to this is glue-making, in which several per cent of dirt might not cause enough trouble to injure the glue seriously. There are no fixed standards for cleanliness to which casein must conform, and the amount of dirt permissible must be judged from experience or by comparison of the sample with standards of acceptable quality. Obviously the methods described are not sufficiently precise to incorporate in a specification for purchase unless a standard can be agreed upon between buyer and seller.

Color

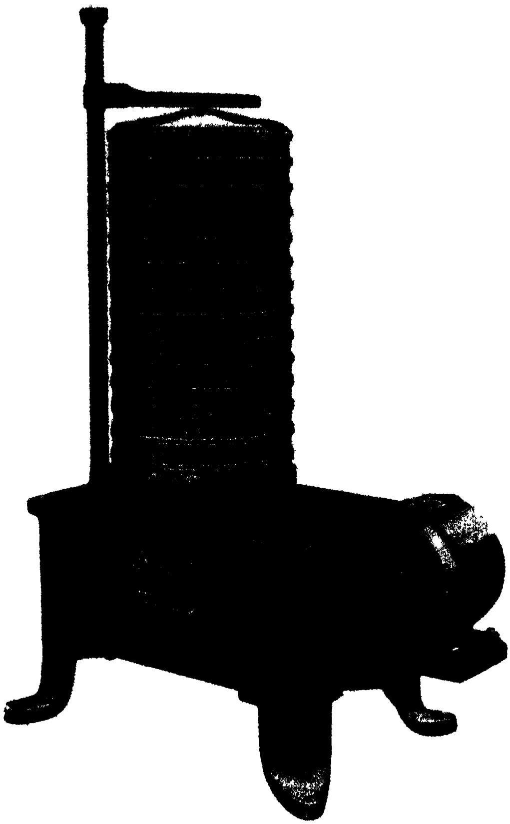
In the case of color also there are no generally accepted standards for casein and no scientific method for its determination. Probably the best procedure is to compare the specimen with standard samples. Even this is not entirely satisfactory because the color of casein changes with time, especially if it is exposed to light, and the yellow color becomes much less noticeable the more finely the sample is ground. Casein of good quality need not be darker than a pale yellow or cream color, and for use in glue-making even a distinct reddish color may not indicate unsatisfactory quality.

Odor

The following test is recommended:[10] "About 10 grams of the casein is soaked in about 10 cubic centimeters of water and an equal volume of rather thick milk of lime added with stirring. After the mixture has stood a few minutes the odor is noted." High-grade commercial caseins should have very little odor, or at most an odor resembling that of sweet milk. A disagreeable odor may not always indicate inferior quality, for the casein may have acquired odors from other materials stored near it during transit. The rancid odor that is frequently considered characteristic of casein is not due to the casein itself but to associated impurities or decomposition products.

Fineness

Casein may be graded for fineness by screening on standard sieves. They should be shaken in standard manner and for a definite length of time. Browne[10] suggests placing a 50-gram sample on a 60-mesh



Courtesy of W. F. Schaphorst and Newark Wire Cloth Co.

Figure 23.—A mechanical sieve shaker for use in determining the fineness of ground casein.

screen and shaking by hand in a horizontal plane at a rate of about 120 strokes per minute. At the end of each stroke the sieve is allowed to strike the palm of the other hand, which is held stationary. At the end of ten minutes the portion passing through is weighed. A machine for doing the shaking mechanically is now on the market. It not only does the work more rapidly and more conveniently but gives more accurately reproducible results.[33]

The test for fineness in casein is particularly important in grading it for use in lime-casein spreaders, in cold-water paints and for glue-making because the rate at which casein goes into solution is governed very largely by the size of the granules. Fineness is also important in the coated-paper industry where the use of too finely ground casein is objectionable because of the possible formation of lumps when the casein is mixed with water.

Solubility

The solubility test is really an empirical test of the ease with which the casein can be dissolved in solutions of limited degree of alkalinity, for which purpose borax is a convenient source of alkali. Practically any casein can be brought completely into solution if the alkalinity is sufficiently increased.

One of the earliest solubility tests to be generally applied to casein[38] consisted in treating 50 grams of casein, ground to pass a 20-mesh sieve, with 300 cubic centimeters of water containing 7.5 grams of borax. When stirred continuously at a temperature of 65°C. solution should be complete in 10 minutes. This test was largely employed to determine the suitability of the casein for coating paper, but it did not tell the whole story and the results required careful interpretation because many caseins that were not completely soluble by this test gave satisfactory results in use.

A test similar to this can be worked out in any industry, or any particular plant, to suit its own special requirements for solvents. Under such conditions it may give valuable indications of the suitability of the casein for the work in question. This is especially true if the character of any insoluble residue is taken into consideration.

An improved borax solubility test has been described by Zoller.[54] Fifteen grams of casein, ground to pass a 40-mesh sieve, is weighed into a 250-cubic centimeter beaker and 100 cubic centimeters of a 0.2 M borax solution (76.32 grams $Na_2B_4O_7 \cdot 10H_2O$ per liter) at a temperature of 30°C. added with vigorous stirring. Allow to stand for 30 minutes, with thorough stirring at intervals of 5 minutes, and frequent stirring during the first 5 minutes. Until familiar with the test use a casein of known qualities for comparison.

This test does not show the suitability of the casein for any commercial work but it does differentiate between caseins made at high and low temperatures. Cooked curd caseins always tend to imbibe water and form a jelly instead of going into solution.

Mummery and Bishop[31] recommend a test for solubility that they consider more significant of the commercial applicability of caseins. Two grams of the casein, ground to pass a 30-mesh sieve, is placed in a ¾-inch test tube, 12 cubic centimeters of borax solution (20.833 grams

$Na_2B_4O_7 \cdot 10H_2O$ per liter) is added and the tube placed in a water bath at 70°C. The casein is stirred thoroughly and at frequent intervals for three-quarters of an hour. If it is not fully dissolved in that time incomplete solubility is demonstrated. The solution is next made up to 100 cubic centimeters with distilled water and one cubic centimeter is pipetted into another test tube together with 10 cubic centimeters of distilled water. To this solution 5 drops of bromthymol blue indicator is added and the color compared with the standard buffer solution made from potassium dihydrogen phosphate and sodium hydroxide according to the Clark and Lubs formula. The pH of this solution, which is called the solubility index, indicates the ease of solubility of the casein. The water used in the test should have a constant pH of 6.

Color	pH	Rating of casein
Blue	Above 6.8	Very good
Green	6.8 to 6.4	Good
Yellow	6.0 to 6.4	Passable
...	Below 6.0	Not soluble

Viscosity

The viscosity of casein solutions may be measured by any of the devices in common use. The Engler viscometer[32] in which the time of efflux through a standard orifice is observed, the MacMichael[55] and Stormer viscometers in which the resistance to the rotation of a submerged cylinder is measured, and the Herschel viscometer[11] in which the rate of extrusion through a standard capillary under pressure is measured have all been used. As pointed out in Chapter 3, such solutions of casein as those in borax are pseudoplastic rather than viscous, but typical casein glues containing calcium hydroxide are truly viscous. For pseudoplastic solutions a single measurement of rate of flow under a constant pressure is inadequate for describing the flow characteristics of the material, several measurements at different pressures being necessary. Nevertheless single measurements are commonly accepted as an arbitrary expression of the "viscosity" of casein solutions for testing purposes.

There is no general standard for viscosity to which all caseins are expected to conform and, in fact, different industries have very different requirements. It is therefore necessary that each industry, or even each individual plant, should work out its own standard method of testing which should be so planned as to give information of value in that particular work. It should also be remembered that the viscosity of a casein solution varies with the kind of solvent used, the pH of the solution, sometimes with the fineness of grinding of the casein, and in some cases at least with details of time, temperature, stirring, etc., in the

preparation of the solution. In glue-making the viscosities of the final mixtures made with different caseins may not agree with predictions based upon the viscosities in solution in borax, for example. In the coating of paper it has not proved possible to determine the viscosity of a casein solution and from the result to predict the viscosity of the coating mixture that will result when clay and satin white are mixed with it. Judgment of the value of the casein must, therefore, be based on the viscosity of the final coating mixture rather than on that of the casein solution.

Under otherwise similar conditions the viscosity of alkaline solutions of casein is directly related to the ash content. In general the higher the content of ash in the casein the higher the viscosity of its solutions.[18, 32]

Strength

The term "strength" as applied to casein means different things according to the various industries in which it is used. In glue work it is used in the sense of the strength of glued joints and it is generally regarded as a property of the glue formula rather than of the casein. The tests applied in the woodworking industry, as described in the chapter on casein glues, are in reality tests of success in correlating glue formula with gluing technic, and at most indicate the suitability of the casein for making strong joints in the particular glue formula and gluing technic used. Low test results suggest that the casein should be used in a different formula or by a different technic, not that it is incapable of making strong joints.

Any test to show the strength of casein should approximate the conditions under which it is actually used. For the paper coating plant this would involve preparing a solution of the casein with the alkalies used locally and adding this in gradually increasing amounts to a known weight of the clay or other pigments employed. After each addition of adhesive a sheet of paper is coated with the amount of coating that is customarily used, dried, and tested.

By starting this test with an amount of casein that is known by experience to be too little, and increasing the amount well beyond that which will probably be necessary, a series of sheets is obtained that varies from extremely weak to unnecessarily strong and in which the ratio of casein to pigment is definitely known. After air drying, these sheets may be tested for strength of coating.

The precautions to be observed in preparing these coated sheets are important if comparable results are to be obtained. The clay or other pigment must be the same throughout any given series of tests. This means not only the same kind but the same lot, because different lots of

clay of the same kind are apt to require somewhat different amounts of adhesive.

The body stock to which the coating is applied must be the same and the coating must be applied to the same side of the sheet each time. Different papers have widely different casein requirements and the two sides of a sheet are seldom alike.

The weight of coating applied should be held within fairly close limits if errors are to be avoided in the subsequent tests. To accomplish this some sort of mechanical device is necessary in order to spread the coating uniformly and at the same thickness. Such devices are available and do excellent work, but in many laboratories the coating is still applied by brushing by hand. An experienced operator can do very good work in this way but it is difficult for an amateur.

With all these variables and precautions to be observed in preparing the coated sheets it is quite obvious that the method may give excellent comparative results in any given plant but that tests from two or more plants cannot be compared with safety.

After the sheets are prepared they must be tested. This is usually done by means of sealing waxes of graded adhesive strength. Each wax is melted on the end, placed on the coated paper and, after cooling thoroughly, is removed by a quick, vertical pull. The surface of the wax and that of the paper are examined and the number of the wax noted which just fails to pull off any of the coating. To be of value this test must be correlated with the average printing results on the same type of paper. For example, if paper that just prints without picking shows no picking on a No. 4 wax and a slight pick on No. 5 wax, this correlates the two tests, and that ratio of casein to clay which will give this wax test on the coated paper is considered as showing the strength of the casein.

Since different types of printing use inks with various degrees of "tack" or pull it is obvious that different casein-pigment ratios will be needed for the different classes of printing. The "strength" of a casein therefore relates to certain definite conditions of coating and printing, and results in two laboratories in different plants are again not comparable.

Foaming

The development of foam during agitation of casein solutions is very objectionable in many uses. Some caseins, particularly those of high acidity, make solutions that develop foam more readily than others. The following method of testing the foaming tendency of casein has been described:[39] Dissolve the casein at 45°C. in enough 10 per cent

sodium carbonate solution to give a ratio of 5 parts of water to one of casein by weight. Let stand two hours for the foam made during solution to break. Mix with 100 grams of a clay, chosen as standard for the purpose, 100 cubic centimeters of water, add 66 grams of the casein solution, and let stand for one hour at 25°C. with occasional gentle stirring. Weigh out 200 grams of the mixture in a 400 cubic centimeter beaker of low form and agitate for two minutes with a disk one inch in diameter, ⅛ inch thick, rotated at 2,700 revolutions per minute with the top of the disk ¾ inch below the surface of the mixture. Let stand one minute and then weigh 100 cubic centimeters of the mixture. The difference between the weights of 100 cubic centimeters before and after agitation is the foam index of the casein. Duplicate tests should check within 10 per cent.

Methods of Proximate Analysis

Nearly all systems of proximate analysis of casein that have been proposed include determinations of moisture, fat, ash, and nitrogen. Most systems also include an "acidity" determination of some kind. Other procedures sometimes included are determinations of sugar, phosphorus, and calcium.

Moisture

According to Shaw[43] the moisture in casein should be determined by drying a 3 gram sample for 5 hours at 98°C. in a partial vacuum. Snyder and Hansen[45] recommend 80°C. for 24 hours. A large double-walled water oven will give fairly good results if the samples are placed in the center, but an electrically heated oven may not be satisfactory if there are considerable variations in temperature at different points within it. Weighings should be made in stoppered weighing bottles, as open dishes have been found to give low results. Browne[10] found that drying in an evaporating dish in an electric oven at 98°C. for 5 hours at atmospheric pressure gave a maximum of 0.47 per cent less moisture than drying *in vacuo*.

Casein, like many other complex organic materials that are hygroscopic, parts with the last traces of moisture reluctantly and reabsorbs moisture readily. Moreover at temperatures near or above 100°C. chemical changes giving off water may proceed slowly. In dealing with any material of this kind the oven used and the technic of handling the samples should be studied carefully to make sure that reproducible results are being obtained.

Gangl and Becker[22] believe that oxidation may account for slow changes in weight of casein in drying ovens and therefore recommend determination of moisture content by distilling a sample of casein suspended in xylene or monobrombenzene.

Ash

The ash left when casein is burned comes chiefly from four sources, inorganic substances not completely removed in washing the curd, calcium phosphate associated with the casein in milk, calcium oxide in the calcium caseinate of milk, and the organic phosphorus in casein. Theoretically the first three should be completely removed in manufacturing casein but practically they are not. If there is enough calcium oxide present all the organic phosphorus remains in the ash in the form of calcium pyrophosphate. Under such circumstances the ash content determined by simple incineration may be called the *total ash* content. If there is insufficient calcium oxide to fix all the organic phosphorus, part of the organic phosphorus is volatilized during simple incineration, and the ash content determined by simple incineration may be called the *proximate ash* content. In practice with caseins containing 2.5 per cent or more of ash the proximate and the total ash contents should be the same. European workers like to express results in the form of the *true ash* content, which is the total ash minus the P_2O_5 equivalent to the average content of organic phosphorus in pure casein. When determining total ash in a casein whose ash may be less than 2.5 per cent, it is customary to add enough calcium oxide in the form of calcium acetate before burning the casein to make certain that all organic phosphorus will be fixed and then to subtract from the result the amount of CaO added.

To determine proximate ash content, weigh a sample of 2 to 3 grams into a silica dish 2 inches in diameter and distribute the sample in a uniform layer over the bottom. Heat over a very low flame until the casein is thoroughly charred, taking care that it does not burst into flame. Caseins of very low ash content may swell greatly during the charring, may overflow the dish if heated too rapidly[10] and may give difficulty with spattering.[45] It should require about an hour to char the casein thoroughly. The dish is then placed in a cold muffle furnace which is then raised to 600 or 650°C. in 2.5 to 3 hours and held there 5 to 6 hours or until a white ash is obtained. With caseins of very low ash content the ash may fuse and enclose particles of carbon that then prove difficult to burn out. Addition of a little ammonium nitrate near the end of the ignition aids in obtaining a white ash.[10] Caseins of high ash content yield a skeleton ash that allows the carbon to be burned out relatively easily.

Platinum dishes should not be used to determine proximate ash content because organic phosphorus may attack platinum. Glazed porcelain dishes are likewise attacked and fix part of the organic phosphorus.[10, 43]

Determination of proximate ash content is an empirical procedure that must be done under carefully standardized conditions if accurately

reproducible results are to be obtained. For that reason analysts commonly prefer to determine total ash content. Proximate ash content, however, is directly related to the viscosity of alkaline solutions made with the casein[13] whereas total ash content is not. For some purposes, therefore, proximate ash content is the more significant determination.

Total ash content may be determined as follows:[35, 43, 45] Weigh a 2-gram sample of casein, moisten it with 5 cubic centimeters of a solution of calcium acetate containing 30 grams of CaO per liter, and let stand 20 minutes. Then place in a muffle furnace, raise the temperature to 600 to 650°C. in 2.5 to 3 hours, and hold there for 5 to 6 hours or until a white ash is obtained. From the result subtract the amount of CaO added in the calcium acetate solution. Dishes of silica, platinum, or porcelain may be used in this method.

The proximate, total, and true ash content of various caseins are given in Table 10 for comparison.

TABLE 10.—Proximate, Total, and True Ash Content of Typical Commercial Caseins

Casein, sample No.	Total ash (%)	Proximate ash (%), determined in dishes of		True ash (%)*	Observer
		Silica	Porcelain		
217 B	2.19	0.38	1.13	0.58	Browne[10]
218 B	2.29	0.38	1.24	0.68	Browne
1 S	2.25	1.68	1.51	0.64	Shaw[43]
7 SH	3.50	1.92	....	1.89	Snyder and Hansen[45]
221 B	2.33	2.00	2.21	0.72	Browne
2 S	2.66	2.45	2.41	1.05	Shaw
222 B	2.61	2.52	2.59	1.00	Browne
3 SH	4.17	2.71	....	2.56	Snyder and Hansen
5 SH	3.45	2.73	....	1.84	Snyder and Hansen
8 SH	3.55	2.99	....	1.94	Snyder and Hansen
2 SH	3.37	3.20	....	1.76	Snyder and Hansen
3 S	3.23	3.22	3.09	1.62	Shaw
6 SH	3.47	3.52	....	1.86	Snyder and Hansen
4 S	4.60	4.20	4.13	2.99	Shaw

* Total ash minus 1.61, the P_2O_5 equivalent of the organic phosphorus in the casein, is recorded as true ash.

Fat

The earliest method of determining fat in casein is that of directly extracting a sample of ground casein with ether.[10, 24] Many analysts felt that the results were too low because the granules of casein, unless exceedingly finely ground, enclose fat that is not extracted by the ether. To obtain more thorough extraction, trituration of the casein with an equal weight of water and then grinding in a mortar with quartz sand before extracting the mixture has been recommended.[29] A number of methods have been developed in which the casein is dissolved and the fat removed either by centrifuging or extracting the solution with ether.

One of these[47, p. 340] is a modification of the Babcock test for fat in milk in which the casein is moistened and then dissolved in concentrated sulfuric acid. Another method that has been widely used[43, 49] is to dissolve the casein in ammonium hydroxide and extract the solution by the Roese-Gottlieb method[3] or to dissolve in hot, concentrated hydrochloric acid and then extract the solution, after cooling, with ether.[9, 18, 20] Other methods based on more or less similar principles have been described.[26, 27, 34, 37, 41]

Where the fat content of the casein is low, the modified Roese-Gottlieb method gives much higher results than direct extraction with ether; but where the fat content is high the results with the Roese-Gottlieb method are only slightly higher than those obtained by direct extraction. The modified Babcock method seems to agree closely with the method of direct extraction. Some comparative results appear in Table 11. Snyder

TABLE 11.—Comparative Results of Three Methods of Determining Fat

Casein, sample No. or description	Content of fat in % by the method of Direct Extraction	Modified Babcock	Modified Roese-Gottlieb	Observer
1	0.37	0.36	1.16	Snyder and Hansen[45]
2	0.27	0.36	0.97	Snyder and Hansen
3	0.22	0.27	0.68	Snyder and Hansen
4	0.13	0.14	1.03	Snyder and Hansen
5	0.34	0.28	1.64	Snyder and Hansen
6	0.29	0.32	1.79	Snyder and Hansen
7	0.32	0.32	1.89	Snyder and Hansen
8	0.18	0.16	1.52	Snyder and Hansen
Grain-curd	0.08	....	1.50	Shaw[43]
Acid-cooked curd	0.20	....	2.35	Shaw
Buttermilk casein	3.79	....	3.92	Shaw

and Hansen[45] find that the high results of the modified Roese-Gottlieb method are due to a white colloid-like substance that passes into the ether solution, cannot be filtered out, and is therefore included with the fat. For that reason they recommend direct extraction with ether for 48 hours, using a sample of casein ground to 100 mesh. Other workers report difficulty with the modified Roese-Gottlieb method in that the second extraction with ether and petroleum ether does not separate properly and tends to form a jelly. The effect of fineness of the sample on the results obtained by direct extraction is shown in Table 12.

The modified Babcock method,[47, p. 340] which is much more rapid than direct extraction, is as follows: Soak 2 grams of casein in 6 cubic centimeters of water in a small beaker and after about half an hour add, with constant stirring, 9 cubic centimeters of concentrated sulfuric acid (specific gravity 1.84). Pour the solution into the Babcock skim milk bottle and wash out the beaker with 5 cubic centimeters of water and 5 cubic centimeters of sulfuric acid. Fill to the base of the neck with dilute

TABLE 12.—Effect of Fineness of Casein on Content of Fat by Direct Extraction

(Data of Snyder and Hansen[43])

Casein, sample No.	Percentage of fat in sample ground and sifted to pass			
	100 mesh	65 mesh	40 mesh	20 mesh
11	0.31	0.28	0.14	0.15
12	0.26	0.26	0.23	0.16
13	0.28	0.22	0.12	0.12
14	0.50	0.29	0.21	0.20
15	0.37	0.14	0.05	0.05

sulfuric acid (4 cubic centimeters water and 5 cubic centimeters concentrated acid), and whirl in the centrifuge for 5 minutes. Fill with dilute acid, whirl 2 minutes and while still hot read the fat on the graduated neck. The reading multiplied by nine gives the percentage of fat in the casein. The secret of success with this method is in getting the concentration of the acid just right; if it is too strong it will char the fat while if it is too weak the casein will not all be in solution and a reading will be impossible.

The modified Roese-Gottlieb method[43] is as follows: Weigh accurately about one gram of the casein into the Roehrig tube, and add 10 cubic centimeters of water. Shake vigorously, but not so as to carry particles of the casein near the top of the tube. Allow the casein to soak for at least 15 minutes. Add 2 cubic centimeters of strong ammonia water, and shake vigorously, again taking care not to carry particles of casein near the top of the tube. Let the tube stand 10 minutes with occasional shaking, then add 10 cubic centimeters of 95-per cent alcohol and shake until the casein is completely dissolved. Add 25 cubic centimeters of ether, shake thoroughly for half a minute, add 25 cubic centimeters of petroleum ether and shake again for half a minute. Allow to stand for about 20 minutes, or until the liquids have separated, and draw off the ether solution as completely as possible, filtering through a pledget of cotton into a weighed flask. Repeat the extraction with ether and petroleum ether, using 15 cubic centimeters of each solvent. Evaporate the combined extracts and dry the residue at 100°C. to constant weight.

Sugar

Weigh 10 grams of the casein into a 500 cubic centimeter wide-mouthed bottle and add 250 cubic centimeters of 50-per cent alcohol. Shake 4 hours in a motor-driven shaking machine, allow to stand for several hours, and decant the solution. Centrifuge the solution if it is turbid and take 10 cubic centimeters of the clear solution for the sugar determination, which is performed according to the method of Bryan,

Given and Straughn.[11a] The per cent of sugar in various caseins is given in Table 13.

TABLE 13.—Content of Sugar in Commercial Caseins

(Data of Shaw[43])

Description of casein	Sugar content (%)
Natural sour casein	trace
Sulfuric acid-cooked curd casein	1.13
Grain-curd casein from buttermilk	0.95
Sulfuric acid-cooked curd casein from buttermilk	0.62
Sulfuric acid-pressed curd casein from buttermilk	0.33
Grain-curd casein, not washed	3.96
Grain-curd casein, well washed	1.43
Grain-curd casein from partly sour skim milk, not washed	5.43
Grain-curd casein from partly sour skim milk, well washed	0.56

Acidity

Various methods of determining the "acidity" of casein have been devised, the methods differing largely according to the purpose the investigator thought the determination should serve. Some sought a measure of the amount of acid precipitant left in the casein by inadequate washing, while others sought a measure of the amount of alkali required to dissolve the casein and bring the solution to a given pH. As a matter of fact neither of these objectives can be attained by the simple methods of volumetric titration that have been used. Adequate determination of alkali consumption would require the charting of a buffer curve by potentiometric titration.[16] If, however, the determination is regarded merely as one of several empirical methods of gauging conformity of casein to a type that has been found desirable for a specific use, the simple volumetric titration proves very useful provided that the procedure in all details is very carefully standardized so that results are reproducible.

In general, two groups of methods for acidity should be distinguished. The older group provides for leaching the ground casein with water,[10, 24, 29, 45] alcohol,[25, 26, 29] or dilute sodium hydroxide[29] and then titrating the filtrate with standard solution of alkali. The result may be called *free acidity*. The second group provides for complete solution of the sample of casein in standard alkali solution and determination of the alkali consumed, either by direct titration or by back titration with standard acid solution.[10, 14, 45, 51] The result may be called *total acidity*.

Total acidity may be determined as follows:[10] Place a 1-gram sample in a flask and run in 25 cubic centimeters of $0.1N$ sodium hydroxide solution from a pipette. Gently agitate the flask during this addition. Then stopper the flask and continue agitation until the solution is complete. Remove the stopper and wash the portion of solution wetting it into the flask with a stream of water from a wash bottle; add 100 cubic

centimeters of distilled water (neutral to phenolphthalein) and back titrate the solution at once with 0.1*N* acid, using 0.5 cubic centimeter of alcoholic phenolphthalein solution (1 gram per 100 cubic centimeters) as indicator. Run in the acid fairly rapidly with vigorous shaking of the flask so as to prevent precipitation of the curd locally. The number of cubic centimeters of 0.1*N* alkali used up by 1 gram of moisture-, fat-, and ash-free casein is called the "total acidity" of the sample.

Pure isoelectric casein under these conditions is said to combine with 9.0 cubic centimeters of 0.1*N* alkali. Commercial casein may have either higher or lower acidity depending on the presence of free acid, proteins other than casein, or decomposition products on the one hand, or lime and other bases on the other hand. There is a close relation between total acidity and proximate ash content; other things being equal, the higher the ash content the lower the acidity.

If this method is to give concordant results several precautions must be observed. The flask must be kept stoppered except when making additions or titrating. The amount of indicator must not vary from that specified, and it must be adjusted with alkali so that one drop added to distilled water does not change its reaction. Local coagulation of casein during the titration must be guarded against, and the total time the casein is in contact with the alkali must not exceed 30 minutes at room temperature.

Snyder and Hansen[45] determine the pH of a 5 per cent suspension of casein in water. Either the hydrogen electrode or the quinhydrone method may be used, the results being in close agreement. The concentration of casein in the suspension and the fineness of the sample within wide limits have no bearing on the result. For a number of different lots of casein the pH ranged from 3.81 to 5.64, all the commercial caseins having pH below 4.6. Snyder and Hansen also determine the electric conductivity of a 5 per cent suspension of casein in conductivity water after standing 17 hours. Results with different caseins ranged from 0.148 to 1.41 times 10^{3} mhos and apparently were not directly related to pH.

Phosphorus

For medium- or high-ash caseins dissolve the ash in 5 to 10 cubic centimeters of concentrated nitric acid on the steam bath; wash into a small beaker and boil to oxidize the phophorus; neutralize with ammonium hydroxide and then make faintly acid with nitric acid. Add 2 grams of ammonium nitrate; heat nearly to boiling and add an excess of ammonium molybdate solution. Digest for an hour at about 65°C. and test the clear supernatant liquid with more molybdate solution to make sure that precipitation is complete. Filter and wash with water or

ammonium nitrate solution and then dissolve the precipitate from the filter with hot ammonium hydroxide. Wash the filter with hot water, collect both the solution and washings in a small beaker until the volume does not exceed 100 cubic centimeters. Nearly neutralize with hydrochloric acid, cool and add slowly and with constant stirring an excess of magnesia mixture. After about 15 minutes add 12 cubic centimeters of ammonium hydroxide (specific gravity 0.90). Let stand for at least 2 hours, preferably over night, filter, wash with dilute ammonium hydroxide until practically free from chlorides, dry, ignite until white or grayish white, and weigh as $Mg_2P_2O_7$.

As explained in connection with the ash determination, unless more base is added some of the organic phosphorus is lost in ashing casein of low ash content. For such caseins the phosphorus should be determined on the ash obtained after treating the casein with calcium acetate solution, or the casein may be treated with magnesium nitrate solution, ignited and the ash dissolved in hydrochloric acid according to the method for total phosphorus in fertilizers.[3] In case hydrochloric acid is used as a solvent add 15 grams of dry ammonium nitrate to the solution before precipitating with ammonium molybdate.

Calcium

Weigh accurately 1 to 2 grams of casein and heat in a crucible until the material is charred. Digest the residue with dilute hydrochloric acid; filter; dry the filter; ignite it and repeat the extraction with hydrochloric acid. Unite the acid extracts and make up the solution to 100 cubic centimeters. To 50 cubic centimeters of the solution add 1 cubic centimeter of concentrated sulfuric acid and heat to boiling. Add 100 cubic centimeters of alcohol, stir well and allow to stand over night. Collect the precipitate in a weighed Gooch crucible, wash it with alcohol until free from acid, dry it at 120°C. and weigh as calcium sulfate, $CaSO_4$. The calcium may also be determined by digesting the casein with aqua regia and then following the customary procedure of precipitating with ammonium oxalate and igniting to calcium oxide.

Nitrogen

Nitrogen is best determined by the usual Kjeldahl method, or by the Gunning method.[3] The digestion should be continued for 6 hours in the Kjeldahl method or for 4 hours in the Gunning method in order to break down all the compounds present in the casein.[45] The percentage of nitrogen found multiplied by 6.38[3, 10] will give the percentage of pure casein in the sample, assuming that casein is the only nitrogenous material present. Since this assumption is not entirely correct, the factor 6.61 has been proposed[24] for computing the fat-, ash-, and moisture-free

material present and the factor 7.70[24] or 7.73[7] for computing the content of commercial casein, except that the latter factors are not correct for buttermilk or rennet caseins. The factor 7.70 is used in calculating the amount of commercial casein in analyzing coated papers.[47, p. 508]

Miscellaneous

The micro-organism count, the reductase test[15] and the Warth incubation test will be found described in the chapter on storage of casein. These tests are of value in determining the conditions of cleanliness during the manufacture of the casein and in selecting caseins that will store well.

Methods of identifying[1, 6, 28, 42, 48] and determining[2, 46, 47, p. 508] casein in coated papers and for detecting casein in wood that has been impregnated or glued with it[37a, 53] are described in the literature but will not be discussed here.

Representative Analyses and Specifications

During 1918 and 1919 many analyses of casein of different types and from various sources were made at the U. S. Forest Products Laboratory for the purpose of preparing suitable specifications for government purchases of casein to be used in making casein glue for Army and Navy aircraft. The results of these analyses and the specifications resulting from them were published.[10, 13, 52] With the subsequent shift from wood to metal in the construction of military aircraft and the development of the manufacture of prepared casein glues by private industry, government purchases of casein became unimportant. Further publications on analyses and specifications for casein have been scattering and often fragmentary.[5, 17, 21, 44, 45, 50] The data of the Forest Products Laboratory, while now old, still present the most adequate picture available of the range in analyses of different kinds of casein and for that reason are presented in Table 14. The great variation in commercial caseins prevailing at that time is revealed clearly, particularly in the important matter of ash content. The present casein market on the whole is believed to be more uniform because of the development of improved methods of production by the larger manufacturers and the wider adoption of the better discontinuous processes by small producers. Some analyses of casein made in more recent years are given in Table 15.

The specification used for government purchases of casein for making casein glue[52] in 1919 contained the following provisions, which were based on the methods of testing described by Browne:[10]

Color: white or light cream
Odor: nearly odorless, with not more than a trace of sourness
Moisture: Not more than 10.0 per cent

Fat: Not more than 1.0 per cent (calculated on moisture-free basis)
Ash: Not more than 4.0 per cent (calculated on moisture-free basis)
Nitrogen: Not less than 14.25 per cent (calculated on moisture-, fat-, and ash-free basis)
Acidity: Not more than 10.5 cc. of 0.1N alkali per gram.

TABLE 14.—Proximate Analyses of Commercial Caseins*

(*Data of Browne*[10] *in 1919*)

Description of casein	Number of samples analyzed	Percentage of— Moisture	Fat	Proximate ash	Nitrogen	Acidity, cc. 0.1N alkali per gram
Lactic acid						
Maximum		9.78	1.44	5.38	14.89	12.5
Average	12	7.70	0.88	3.46	14.38	9.7
Minimum		6.21	0.21	2.08	13.62	5.1
Grain curd						
Maximum		8.25	0.77	2.87	14.94	9.7
Average	7	6.84	0.50	2.78	14.71	8.9
Minimum		5.92	0.28	2.53	14.45	6.8
Sulfuric acid, pressed curd						
Maximum		10.18	2.48	5.34	15.32	13.9
Average	23	7.97	1.41	3.92	14.42	10.4
Minimum		6.23	0.15	2.62	13.22	5.7
Hydrochloric acid, pressed						
Maximum		8.60	1.15	6.10	14.98	10.7
Average	14	7.37	0.56	4.52	14.39	8.7
Minimum		4.86	0.00	3.14	13.83	6.7
Acid, cooked curd						
Maximum		10.89	1.42	4.93	14.76	9.6
Average	18	8.16	0.65	4.45	14.40	7.5
Minimum		6.96	0.22	3.79	13.94	5.8
Rennet						
Maximum		10.32	0.83	8.39	15.10	11.3
Average	3	8.29	0.63	7.97	14.41	7.9
Minimum		5.54	0.39	7.18	13.82	5.5
Buttermilk						
Maximum	..	8.97	23.32	2.18	15.06	10.0
Average	9	6.97	9.56	1.36	14.77	9.2
Minimum		5.33	3.79	0.88	14.42	8.5
Lactic from Argentina						
Maximum		9.28	2.11	4.30	14.88	13.5
Average	8	8.20	1.10	3.87	14.66	11.5
Minimum		5.94	0.53	3.62	14.30	9.0
Pure caseins:						
Hammarsten method		10.02	0.00	2.73	15.50	6.5
Hammarsten method		7.42	0.22	0.95	15.58	8.5
Van Slyke method		5.70	0.19	0.24	15.20	8.2
Van Slyke method		9.27	0.13	0.38	15.00	7.9
Van Slyke method		9.41	1.66	0.38	15.33	8.1

* Fat determined by direct extraction and expressed on the moisture-free basis. Ash expressed on the moisture-free basis. Nitrogen expressed on the moisture-, fat-, and ash-free basis.

TABLE 15.—Proximate Analyses of Commercial Caseins
(Data of 1931 to 1934)

Observer and description or sample no. of casein	Number of samples analyzed	Percentage of					Acidity			Strength test (for coating paper) (grams)	Viscosity of 9% solution in NaOH at pH 9.1 (centi-poises)	Borax solubility test
		Mois-ture	Fat (direct ext'n)	Proxi-mate ash	Total ash	Nitro-gen	Total (cc.)	Free (cc.)	pH			
Bell & Gould[5]												
1	1	5.91	0.27		1.93		8.0	1.0	4.74	7	14.09	excellent
2	1	7.22	0.00		2.68		8.5	5.6	4.60	7	7.51	good
3	1	7.86	0.18		3.89		7.0	6.4	5.00	9	14.73	thick
4	1	8.03	0.18		3.76		7.0	6.2	5.03	11	12.22	thick
5	1	8.35	2.70		3.90		6.0	5.0	5.20	9	13.88	slowly sol.
6	1	7.48	0.45		5.11		6.2	7.8	5.14	12	8.57	thick
7	1	6.23	0.18		3.63		10.2	13.0	4.32	11	14.36	fair
8	1	6.58	1.08		3.05		10.5	14.2	4.25	12	11.36	slowly sol.
9	1	6.30	0.09		3.39		9.6	15.2	4.35	11	11.66	poor
10	1	6.49	0.00		4.34		10.2	16.2	4.46	12	17.40	very insol.
Theophilus[50]												
Idaho	65	4.53	0.19		3.76	14.66	14.2	10.7				
Snyder and Hansen[45]												
Idaho	8	4.83	0.27	2.85	3.98	14.15	14.3	10.6	4.72			
Smith[44]												
Lactic	3			2.57				13.7	4.08			
Grain curd	1			2.78				8.9	4.5			
Sulfuric	3			3.23				9.3	4.24			
Hydrochloric	1			2.76				10.6	3.68			
Sheffield	2			1.34				0.9	4.74			
Argentine	1			2.02				8.2	4.26			

References

1. Anonymous, *Paper Makers' Monthly J.; Papeterie*, **42**, 122 (1920)
2. Anonymous, *Paper Ind.*, **8**, 264 (1926) ; *Pulp and Paper Mag. of Can.*, **24**, 572 (1926)
3. Association of Official Agricultural Chemists, "Official and Tentative Methods of Analysis," Superintendent of Documents, Washington, D. C.
4. Baum, F., *Chem. Ztg.*, **52**, 517 (1928)
5. Bell, R. W. and Gould, S. P., *J. Dairy Sci.*, **14**, 337 (1931)
6. Bentzen, T. C., *Paper Mill*, **52** No. 21, 20 (1929) ; *Paper Trade J.*, **88** No. 23, 59 (1929)
7. Bogue, R. H., *Chem. Age.*, **30**, 103 (1922)
8. Bradford, H., *Paper Mill*, **56** No. 8, 2 (1933) ; see also *Oil Paint & Drug Reporter*, **123**, 15 (Jan. 23, 1933)
9. Bray, T. and Major, F., *J. Soc. Chem. Ind.*, **42**, 106 (1923)
10. Browne, F. L., *J. Ind. Eng. Chem.*, **11**, 1019 (1919)
11. Browne, F. L. and Brouse, D., "Colloid Symposium Monograph," **5**, 229, New York, Reinhold Publishing Corp., 1928

11a. Bryan, A. H., Given, A., and Straughn, M. N., *U. S. Dept. of Agric. Circ.* 71; *J. Ind. Eng. Chem.*, **3**, 486 (1911)

12. Burr, *Milch. Zentr.*, **6**, 385 (1910)
13. Butterman, S., *J. Ind. Eng. Chem.*, **12**, 141 (1920)
14. Chollet, A., *Lait*, **8**, 21 (1928)
15. Chollet, A. and Camus, A., *Lait*, **13**, 362 (1933)
16. Clark, W. M., *J. Ind. Eng. Chem.*, **12**, 1170 (1920)
17. Davies, W. L., *Paper Makers' Monthly J.*, **67**, 279 (1929) ; *Ind. Chemist*, **5**, 202 (1929)
18. Delorme, J., *Lait*, **15**, 36 (1935)
19. Fouassier, M., *Bull. soc. hyg. aliment.*, **16**, 218 (1928)
20. Fouassier, M., *Lait*, **15**, 386 (1935)
21. Friedel, *Milchwirtschaft. Ztg.*, **34**, 1680 (1929)
22. Gangl, J. and Becker, F., *Milchw. Forsch.*, **16**, 325 (1934)
23. Hevesi, A., *Gelatine, Leim Klebstoffe*, **3**, 43 (1935)
24. Höpfner, W. and Burmeister, H., *Chem. Ztg.*, **36**, 1053 (1912)
25. Höpfner, W. and Jaudas, K., *Chem. Ztg.*, **49**, 281 (1925)
26. Höpfner, W. and Jaudas, K., *Chem. Ztg.*, **50**, 325 (1926)
27. Jephcott, H. and Ratcliffe, N., *World's Dairy Congress Proc.*, **2**, 2171 (1923)
28. Kitching, A. F., *Analyst*, **47**, 206 (1922)
29. Marcusson, J. and Picard, M., *Chem. Ztg*, **51**, 104 (1927)
30. Milham, E. G., *Paper Trade J.*, **88** No. 12, 55 (1929) ; see also *Paper Trade J.*, **94** No. 21, 23 (1932)
31. Mummery, W. R. and Bishop, F., *Analyst*, **55**, 367 (1930)
32. Nabenhauer, F. P., *Ind. Eng. Chem.*, **22**, 54 (1930)
33. Newark Wire Cloth Co., *Drugs, Oils and Paints*, 81, Feb. 1937
34. Pien, J. and Bergier, M., *Lait*, **12**, 640 (1932)
35. Pien, J. and Herschdoerfer, S., *Lait*, **13**, 1081 (1933)
36. Porcher, Ch. and Brigando, J., *Ann. fals.*, **22**, 153 (1929)
37. Ratzlaff, *Milch. Ztg.*, **32**, 65 (1903)

37a. Rendle, B. J. and Franklin, G. L., *J. Soc. Chem. Ind.*, **55**, 105T (1936)

38. Reuter, *Papier-Ztg.*, **32**, 3374 (1907)
39. Richards, C. W. and McFail, L. W., *Paper Trade J.*, **97** No 1, 29 (1933)
40. Roth, F., *Le caout. et la gutta percha*, **23**, 13272 (1926)
41. Schoonjans, A., *Lait*, **5**, 782 (1925)

42. Schulze, B. and Rieger, E., *Papier-Ztg.*, **32**, Tech. wiss. Teil 245 (1934)
43. Shaw, R. H., *J. Ind. Eng. Chem.*, **12**, 1168 (1920)
44. Smith, R. W., Jr., *Ind. Eng. Chem.*, **26**, 819 (1934)
45. Snyder, R. S. and Hansen, H. C., *Ind. Eng. Chem., Anal. Ed.*, **5**, 409 (1933)
46. Sticht, G. A., *Chemist-Analyst*, **17**, No. 4 (1928)
47. Sutermeister, E., "Chemistry of Pulp and Paper Making," New York, John Wiley & Sons, Inc., 1920
48. Sutermeister, E. and Porter, L. W., *Paper Trade J.*, **90** No. 12, 73 (1930)
49. Tapernoux, A., Desrante, R., and Bineau, J., *Lait*, **8**, 592 (1928)
50. Theophilus, D. R., Hansen, H. C. and Snyder, R. S., *Concentrated Milk Ind.*, **2**, 16 (1931)
51. Ulex, H., *Chem. Ztg.*, **49**, 641 (1925) ; **54**, 421 (1930)
52. U. S. Navy Bureau of Construction and Repair, *Aeronautical Specification*, **85**, Jan. 15, 1919 (abstracted in reference 7, p. 325)
53. Whitehead, T. H., *Ind. Eng. Chem. Anal. Ed.*, **5**, 150 (1933)
54. Zoller, H. F., *J. Ind. Eng. Chem.*, **12**, 1171 (1920)
55. Zoller, H. F., *J. Gen. Physiol.*, **3**, 635 (1921)

Chapter 6

Storage of Casein

Albin H. Warth

Commercial casein is a commodity that requires much of the attention given to a food product in the way it is handled in packing and in storage. It is not more perishable than a dry food product, but requires far more attention to storage conditions than does gelatin glue if it is to be kept free from infestation by mites, vermin, and rodents. Therefore prime consideration must be given to the place and conditions under which it is stored, especially if it is to be kept for an extended period of time, such as six months or more.

Life of Product

Casein that has been properly prepared with regard to cleanliness may be kept for two and a half years, or over two successive summer seasons, provided it has been packed in clean, closely woven, burlap bags or multiply paper bags, and has been properly stored.

Warehouse Conditions

The warehouse in which casein is stored should be clean and dry, and maintained at a temperature below 80°F. The floor should be sufficiently elevated to prevent danger of ground moisture, and it should be swept frequently and kept in clean condition. If packaged casein is piled on skids, the latter should be whitewashed or painted with cold-water paint, and the walls should also be kept painted. It is essential that the warehouse room receive ventilation, but such ventilation, if from windows, should be through screens, and the entrance door should also be screened.

Where large quantities of casein are stored in the original bag or sack containers, it is important to separate shipments into different lots and pile the lots apart from one another. If possible these piles should be so arranged as to insure the best ventilation from the source of fresh air supply. When there is little or no circulation of dry air in a warehouse, foul air pockets will build up in the pile or in the proximity of certain of the piles. Measurements of temperature and relative humidity of the piles show discrepancies that can be accounted for only by lack of circulation. Table 16 illustrates the wide variations possible.

TABLE 16.—Variation in Temperature and Relative Humidity in Piles of Casein in Storage

Date	Temperature (°F.)				Relative Humidity (%)			
	Pile 1	Pile 2	Pile 3	Pile 4	Pile 1	Pile 2	Pile 3	Pile 4
March 21	64	63	60	63	66	65	63	74
March 23	54	54	53.5	54	48	48	53	48
March 24	53.5	53	54.5	53	76	75	65	75

Of these piles numbers 1 and 2 received a little ventilation from one door and this air was not dry. The other piles received less ventilation. A relative humidity of 65 per cent or below is best for the storage of casein and air that has been dehumidified by regular dehumidifying equipment is ideal, especially if large tonnages are to be stored. Where the loading or removal of bags is conducted from the front end of a warehouse the storage is best located in the rear. At this end an exhaust fan blower may be placed and kept operating except when outside atmospheric conditions are unfavorable.

Even under ideal warehouse conditions it is unsafe to assume that any quality of casein can be favorably stored. One questionable lot of casein placed in the proximity of a lot of exceptional purity may cause the ruin of the good lot. The purity or cleanliness of the casein has a definite relation to the life of the product, as will be pointed out later; it is therefore evident that, aside from examining a casein for its solubility and strength, a knowledge of its purity is essential. Casein prepared from whole milk instead of skim milk, from dried curd that has become mildewed, or from improperly washed or dried curd may have very poor keeping qualities.

The importance of observing minor details concerning storage should not be overlooked, because casein, when it becomes infested, is subject to such rapid spreading of the infestation that serious losses are incurred, especially where casein is stored in carload lots. Large users as well as suppliers have at times experienced losses of that kind amounting to many thousands of dollars.

Packing Methods

American manufacturers for a long time failed to appreciate the significance of details in the packing of casein, although for many years Argentine casein has been imported in packages that are nearly ideal. Argentine casein is packed in closely woven burlap bags holding 60 kilograms, and tightly stitched to avoid folds and crevices. Domestic

caseins are now being packed in closely woven sacks averaging 125 pounds gross, and measuring 3.7 cubic feet in contents. Multi-ply paper bags are also in use. Under no circumstances should casein be packed in second-hand bags, even though they are washed and renovated, nor should casein be packed in coarsely woven new bags of any kind.



Courtesy of Land O' Lakes Creameries, Inc.

Figure 24.—Sacks of casein in storage.

This applies particularly to the ground product. It is always best to double-stitch the bags with stout twine.

Bin Storage

Large users of casein occasionally find it necessary to empty casein upon receipt into a bin, from which it is withdrawn as required. There are commercial advantages in handling casein in this way, and often bin storage is used for the purpose of blending two or more varieties. While this method of handling has advantages it cannot be wholly recommended because the product may become infested with insects without sufficient warning to permit checking the infestation except by drastic means.

If it is necessary to employ bin storage, the bins should be constructed with sloping side walls so that the casein will not pocket and will be kept freely moving. Bins are best made from tongue and groove maple, if wood is used, or they may be sheeted with metal, such as charcoal tin-plate, when built with softwood. They may be filled from openings in the floor above, and the casein removed by means of a screw conveyor located at the foot of the sloping bottom of the bin.

Infestation

Casein is subject to infestation by molds, bacteria, insects, and rodents. The last are often a serious annoyance because they gnaw the bags, spilling the contents and subjecting it to the danger of insect infestation. They also occasion losses by what they actually consume and by making the spilled casein so dirty that it is not fit for use.

Molds

Casein is most often contaminated by mold spores while in the state of moist curd during manufacturing, but any casein that has become slightly moist or wet furnishes a good food supply for all types of mold. Since the air is filled with spores of various kinds it is easy for them to gain access to stored casein, and if sufficient moisture is present they will immediately germinate and multiply. If casein is kept dry, spores will not develop; and if it is free from mold when packed it will not become moldy in storage unless it becomes moist and is infested by spores from without. A wet bag of casein, or one that shows signs of having been wet in transit, should never be stored in the same warehouse room or bin with other lots of casein.

The number of molds per gram of casein varies greatly according to the conditions of manufacture; a California casein possessing a strong sour odor contained 500, while a natural soured casein contained 1,000 molds per gram. The molds were mostly of common types such as mucor, aspergillus, and penicillium, all of which cause decomposition of casein.

Moths

By far the most common and dangerous infestation of casein is by a small moth. At first little was known concerning the nature of this moth and it was a constant source of annoyance to both producers and consumers of casein. Its ravages have at times been the cause of the loss of an immense tonnage of casein in the United States.

E. A. Back, Entomologist in Charge of Stored Product Insect Investigations, U. S. Bureau of Entomology, Washington, D. C., has identified the moth commonly infesting casein as the webbing clothes moth, *Tineola bisselliella.* He says that it is peculiar that this pest, which normally feeds upon woolens and furs, should also infest casein. Mr. Back further says that a thorough fumigation with hydrocyanic gas will prove very satisfactory as a control measure.

Frank E. Lutz[1] describes the moth as follows: "*Tineola bisselliella* has a webbing larva; it makes no case but feeds naked, usually in a fold or crevice of the material it is eating, and often under the web of silk that it spins wherever it goes. The cocoon is an irregular affair of silk and food material, somewhat resembling the case of pellionella, another

species of clothes moth. The adult is about the size of pellionella. The front wings are yellow and without spots; the hind wings are pale."

The other species of moth known to infest casein is *Tinea pellionella*. *Tineola biselliella* is the commonest of the clothes moths from Pennsylvania southward, while in the northern portion of the country *Tinea pellionella* is much more common. This latter moth is darker in color than the former and the larvae spin movable cases in which they travel about. In the warmer parts of the country it is many brooded, and the processes of generation go on continuously, while in the north cold weather arrests them.

When these insects manifest themselves in commercial casein they are often found in quantities, clinging to the inner side of the burlap bags, and also at the top and bottom of the product. If one bag in a warehouse becomes infested all bags in the proximity are also likely to become infested, because the full-grown moth, freeing itself from the mesh of the bag, will fly to another bag, deposit its eggs, and the larvae resulting will go through the cycle to the adult moth.

Beetles

Several kinds of beetles and their larvae are known to infest casein. They are numbered among the "stored grain" and "leather" pests. The more common of the "stored grain" pests which infest casein are the cadelle (*Tenebrioides mauritanicus*), the foreign grain beetle (*Cathartus advena*), and the confused flour beetle (*Tribolium confusum*). Of the "skin-devouring" species which infest casein the larder beetle (*Dermestis lardarius*) and the leather beetle (*Dermestis vulpinus*) are undoubtedly the most common.

The cadelle is a slender, flattened, black beetle, about one-third of an inch long. It resembles the meal worms in appearance, but is smaller and the larvae are dirty or chalky white in color and relatively longer than those of similar beetles. The foreign grain beetle is a small, reddish-brown beetle, and because of its relatively small size it often remains undiscovered in the warehouse. It feeds mostly on mold, to which it is readily attracted. It does not infest casein unless the latter has become moldy and it will not inhabit a place that has been well whitewashed. The confused flour beetle is often a serious pest. It is a slender, dark-brown beetle, scarcely one-sixth of an inch long. Instances are known where these beetles have infested casein to such an extent that they have literally covered the bags on the outside and the burlap resembled the top of a pepper shaker because of the holes where they emerged.

Dermestidae are usually oval, plump, dark beetles, less than three-eighths of an inch long. In *Dermestis vulpinus* there are two characteristic white spots below the last abdominal segment. *Dermestis*

vulpinus is more apt to be encountered in the south than *Dermestis lardarius*. None of the beetles does nearly so much damage as the larvae of moths.

Biological Tests and Standards

Biological tests have been neglected to a sad degree in determining the cleanliness in the preparation of so important a commodity as casein, and much more attention should be paid to them. This field has been less neglected by the gelatin glue makers, and when followed is capable of yielding great economic savings. Biological tests on casein are of importance to the producer, seller and purchaser, as a means of establishing quality grades, as well as preventing subsequent deterioration of the quality of the casein stored. Caseins may be judged biologically by both the microörganism count and by the incubation test, and standards established that will insure good commercial quality and satisfactory operating results in all the various lines of industry that use casein.

In formulating biological standards for casein, consideration may be given to the specific use to which the casein is put. In the manufacture of pastes, food products, composition cork, cold-water paints, casein latex compositions, etc., it is quite essential to hold to a high standard for the purpose of maintaining uniform quality of production. In the manufacture of casein plastics it is not so essential; and in mills where the casein is washed, as in certain lines of coated paper work, the impurities are more or less removed and the washed product may conform to its required standard.

The following standards are drawn for such commercial caseins as are used in pastes, adhesives, and coatings of the higher grade. They are also applicable to food caseins and to grades of casein that are to be kept for a long time.

Caseins containing 100 or less molds per gram, but decomposing in 24 hours under incubation are rejectable.

Caseins containing more than 100 molds per gram, even though decomposition in incubation does not set in within 72 hours, are rejectable.

Caseins containing larvae, webs, or moths of any species are rejectable.

Caseins acceptable for storage are those that contain 100 or less molds per gram and that do not decompose within 72 hours.

Microörganism Count

A quantitative estimation of the number of microörganisms in casein may be made by colony enumeration in plates of agar. In conducting this test it is absolutely necessary that all glassware used shall be sterile. This may be assured by keeping it at a constant temperature of 150°C. for one hour in an oven. After all glassware has been sterilized, 10 grams of casein is weighed out and enough boiled, cooled, distilled water

added to make up to a volume of 100 cubic centimeters in a sterile bottle. After thoroughly mixing, shake the bottle at least 25 times, then withdraw 1 cubic centimeter of the supernatant liquid with a sterilized pipette and deliver it into a sterilized Petri dish, 10 centimeters in diameter. To the liquid in the Petri dish add 10.0 cubic centimeters of standard nutrient agar solution at a temperature of 42°C. After tipping the dish back and forth a few times to mix the contents allow the agar to solidify in a horizontal position, transfer the Petri dish to an incubator as soon as the agar is hard, and incubate the culture in a dark, well ventilated incubator where the atmosphere is saturated with moisture. At the end of 24 hours count the colonies with a lens that magnifies five diameters. The number of colonies counted, multiplied by ten, gives the number of microörganisms (bacteria or yeasts) contained in 1 gram of the sample of casein. If the microorganisms are molds they will require several days for development.

Warth Incubation Test

A 200 cubic centimeter Florence flask, and a 4-gram plug of cotton on a watch glass are placed in a drying oven for one hour at a temperature of 150°C. in order to sterilize both flask and cotton. The plug must be of sufficient size to supply enough friction when placed in the mouth of the flask to permit the latter to be lifted by the plug.

Fifteen grams of the sample of casein is placed in the flask, and 90 cubic centimeters of recently boiled and cooled distilled water is added. The flask is stoppered with the cotton plug and, without shaking the contents, is put in an oven maintained at a constant temperature of 37.5°C. It is left in the oven until putrefaction sets in, as is evidenced by a distinctly disagreeable odor often accompanied by fungus growth. The plug of cotton may be taken out at intervals in order to note the progress of putrefaction. A good product will stand up well for at least 48 hours and will show no signs of marked decomposition under 72 hours.

A casein that holds up for 72 hours is one that may be stored in a dried state for more than a year, or even two years, without deterioration of its chemical or physical properties. Caseins that "go bad" in the test are generally those that have been insufficiently washed, insufficiently dried, or otherwise improperly prepared. Such caseins can be stored only with considerable risk of deterioration and insect infestation, which may occur within the course of a very short time.

Table 17 reports the results of examination of a number of samples of casein by the Warth incubation test. Between 1915 and 1925 nearly 50 per cent of the samples examined by this test were rejectable. Of domestic caseins 60 per cent and of Argentine 45 per cent were rejectable. Both the French and the New Zealand caseins gave better ratings. Between 1925 and 1938 the status with respect to cleanliness

TABLE 17.—Typical Results of the Warth Incubation Test

Sample No.	Kind of casein		Time required to putrefy (hrs.)	Remarks
2300	Domestic,	Hydrochloric	36	Putrefaction
3023	"	Hydrochloric	16	Putrefaction
23659	"	Hydrochloric	72	Mold formed, but only slight amount of putrefaction
24148	"	Hydrochloric	48	In 72 hours very putrid
2284	"	Sulfuric	over 72	No change in 124 hours
2285	"	Sulfuric	over 72	Very slight change in 124 hours
2301	"	Sulfuric	over 72	Held 110 hours
2312	"	Sulfuric	36	Putrefaction
20197	"	Sulfuric	over 72	Lactic acid 1.18 per cent
16051	"	Sulfuric	over 72	Somewhat musty at 114 hours
9751	"	Lactic	72	Decomposed in 96 hours
23658	"	Lactic	72	Mold formed at 48 hours
26101	"	New process	72	Odor of cheese
19890	"	Grain curd	72	Putrefaction
3843–9	"	(5 samples)	60	Putrefaction
10628	Argentine,	Hydrochloric	24	Moisture 9.3 per cent
3556	"	Lactic	over 72	Held 96 hours. Bacterial count less than 100 per gram
10641	"	Lactic	24	Moisture 10.2 per cent. Mold spores 9,000 per gram
14973	"	Lactic	48	Original sample had musty odor
15063	"	Lactic	60	Badly decomposed
15164	"	Lactic	over 72	Original sample very clean
16564	"	Lactic	24	Moisture 11.7 per cent
17667	"	Lactic	over 72	Moisture 8.7 per cent
2312	French,	Lactic	over 72	No change in 110 hours
20219	"	Lactic	72	Putrefaction; moisture 10.0%
20624	"	Lactic	over 72	Lactic acid 0.72 per cent
22493	"	Lactic	over 72	No change in 92 hours
15101	New Zealand,	Lactic	40	Unclean bags developed moths
15267	" "	Lactic	48	Developed moths in 6 months
15274	" "	Lactic	over 72	Slight change in 72 hours
20022	" "	Lactic	over 72	Lactic acid 0.40 per cent
2283	British Indian		72	Mold fungus

of domestic caseins and their behavior in the incubation test improved materially so that approximately 55 per cent passed the incubation test satisfactorily.

Bacterial Count vs. Incubation Test

The time required for a casein to fail in the incubation test is not necessarily a function of the number of bacteria in the sample, even when only one general type of casein, such as lactic acid casein, is considered.

Table 18 by C. L. Ewing gives the bacterial count per gram and the incubation tests on four samples of casein. The type of bacterium plays a more important part in the decomposition of casein than does the actual number. A casein that will stand up only from 24 to 48 hours on incubation is generally one that contains a large number of a type of liquefying or decomposing organisms. The presence of 10,000 bacteria in a gram of casein does not indicate any serious contamination because milk normally contains that many or more.

TABLE 18.—A Comparison of Bacterial Count with the Incubation Test

(Data of C. L. Ewing)

Sample No.	Bacteria per gram (approx.)	Incubation test: Time required for decomposition (days)
1	4,000	10
2	4,000	6
3	2,000	7
4	10,000	10

Changes in Casein During Storage

An Argentine lactic casein stored in a closed 60-kilogram sack in a dry warehouse at a temperature ranging between 15° and 30°C. showed a loss in viscosity from 55 seconds to 50 seconds in the course of 18 months and a corresponding loss in glue strength.* A New Zealand casein, which in 6 months' storage became infested with moths, showed a decrease in viscosity from 58.5 to 41 seconds. The viscosity of both of these caseins could be increased by sifting out the fine casein dust resulting from the deterioration of the product. The viscosities here referred to were determined by means of an Engler viscometer, on a 10 per cent solution of a sample dissolved in water with 15 per cent of its weight of borax. The times of flow are for the discharge of 100 cubic centimeters of the solution at 70°F.

The following viscosities and glue strengths were obtained on Argentine lactic caseins held in storage for a year and a half, the stock being fumigated by sulfur dioxide after deterioration had set in:

Sample No.	Viscosity	Glue strength
484	37.8 sec.	795 lbs. per sq. in.
495	42.0 "	975 " " " "
413	45.5 "	932 " " " "

The original standard for the three lots was 45 seconds viscosity and 1,000 pounds per square inch glue strength. Sample 484 was found to

* For the limited significance of "glue strength" tests of a casein in this connection consult Chapter 5.

have become both rancid and sour, while 495 and 413 were perfectly normal in chemical composition.

The amount of borax required to neutralize and dissolve a casein bears a definite relation to the age and condition of the casein. This, and also the loss of strength that casein undergoes when subjected to unfavorable storage conditions, is shown by the following storage records for four samples:

In October 1911 four samples were stored in a room that was warm in winter and hot in summer. Samples A and B were full bags but of unknown kinds; samples C and D were small lots in cloth bags, C being a domestic sulfuric-acid, and D a domestic hydrochloric-acid casein. They were tested for the amount of borax required to give a neutral solution and for the amount of casein required to give a strong coating with 100 parts of clay when applied to paper. The results of tests before and after storage appear in Table 19. In taking the samples for tests from A and B the outer portion in the bags was rejected where it had become wormy but because of the small size of lots C and D this could not be done and they were thoroughly mixed before sampling. All lots appeared in good condition in October 1911 and in April 1912, but worms had made their appearance in October 1912.

A Scandinavian casein stored in June 1911 and examined in the winter of 1912-13 lost 3.5 per cent of its weight in the form of webs and adhering casein when it was screened on a 15-mesh sieve. The strength test showed that 10 parts of the sifted casein were equal to 11-12 parts of the unsifted.

TABLE 19.—Effect of Storage on the Consumption of Borax and the Strength of Casein in Paper Coating

Date	Percentage of borax required to give a neutral solution				Grams of casein required per 100 grams of clay for coating paper			
	Sample A	Sample B	Sample C	Sample D	Sample A	Sample B	Sample C	Sample D
October 1911...	10	17	7.5	6.5	7	8–9	8	7
April 1912...	9.5	16	6	5.5	8	8–9	8	7
October 1912...	7.5	14	6	4	10	12	8-9	8–9
April 1913...	...	...	...	...	8–9	12	9	8–9

An unground domestic lot was screened and 7 parts of that retained on the 15-mesh sieve was found to have as much strength as 12 parts of the portion passing through the wire. Another domestic casein gave strength tests of 10 parts of the retained to over 22 parts of the sample

passed by the screen. In the latter case the solution was almost water-thin and contained considerable insoluble matter.

In a single lot of casein that tested 9 parts per 100 parts of clay in October 1910, the part retained by the screen in the winter of 1912-13 tested 10 parts per 100 parts of clay while the portion passed by the screen required 24 parts. This was for the top bag in the pile, which had become badly infested with moths. A bottom bag, which was not infested, was tested at the same time and the strength found to be 8 parts for the retained, and 9 parts for the portion passed by the sieve. During this same period other lots of casein were stored in another building, which was not heated even in winter, and which was fairly cool in summer. These lots showed no infestation and no decrease in strength. Other lots were stored in warehouses on "Black Tom Island" in New York between July 1911 and August 1913. In June 1913 they had lost very little of their original strength and showed very little infestation with worms, but by August 1913 most of the lots were badly infested. This evidence seems to be conclusive as to the damage done by insects and the time of year in which it occurs. It should also be noted that all observations were made in New York or points farther north and that in warmer climates the damage would be still greater.

Fumigation

It is generally inadvisable to fumigate packaged casein unless there is a real necessity for doing so. It is, however, necessary to check immediately an insect infestation when it first sets in. In cold climates infestation by moths may be checked by placing casein out of doors in freezing weather. If checked in this way, the bags should be swept off, placed under cover and the contents sifted and transferred to new bags if infested to any considerable extent.

In most instances of insect infestation, it is necessary to employ a fumigant such as hydrocyanic acid or sulfur dioxide. On account of the toxic dangers in the use of hydrocyanic acid for fumigation, except under exceedingly careful control, it is expedient to use sulfur dioxide.

To check the infestation of casein in bins or broken packages the material may be sprayed with carbon tetrachloride. This treatment is especially effective for casein that has become infested by beetles of the larger sort such as *Dermestis vulpinus*. When such a spray is used the insects will crawl to the top and then the casein may be covered by clean burlap saturated with a highly diluted solution of hycol. This double treatment will kill insects and permit their easy removal.

Use of Sulfur Dioxide

When using sulfur dioxide fumigation, doors and windows must be sealed by cloth strips or otherwise before the sulfur dioxide gas is set off

in the storeroom. The size of the room is first estimated in cubic feet. One pound of gas per 100 cubic feet is sufficient to destroy moths and larvae, provided the room is left undisturbed for not less than 18 hours. Where a cylinder of sulfur dioxide is used the valve may be opened so as to allow gas to flow freely but it is best not to open the valve wide. Either a partially empty large cylinder or a small cylinder may suffice for the size of the room.

Following the treatment of stored casein with sulfur dioxide the storeroom must be left open and thoroughly aired. Under these conditions sulfur dioxide will not permanently injure the casein in any way. It may, however, be necessary to brush off the bags, or even to sift the product and repackage in fresh burlap bags to clean it thoroughly.

The method followed at casein manufacturing plants of simply sifting it through a coarse mesh to remove webs, and a fine-mesh sieve to remove powder, will not permanently eliminate danger of reinfestation unless a process of fumigation is also resorted to.

Use of Sodium Cresylate

Sodium cresylate is an excellent material for spraying bags of casein where it is necessary to resort to the use of liquid germicides. The sodium cresylate is made up as follows:

1 quart U.S.P. cresol
1 quart 50-per cent caustic soda
1 quart water.

It does not have the objectionable odor of phenol, cresol, formaldehyde or of many products found on the market. One part of the base is diluted with 50 parts of water. It can be used in the ordinary spray gun made for liquid plant insecticides and used freely and with safety for spraying the exterior of the bags.

Reference

1. Lutz, Frank E., "Fieldbook of Insects," New York, Putnam Publishing Co.

Chapter 7

Casein Plastics

George H. Brother

Casein plastic, as an industrial material, has not been utilized in the United States as much as its characteristics seem to warrant. It has been applied neither as extensively nor as intensively as in Europe, despite the facts that a much greater excess of raw material (skim milk) is available and that the American people are probably the most plastic-conscious people in the world.

There are a number of reasons for this difference in development. One of the most important is the fact that the first attempts to produce casein plastics in the United States followed indiscriminately the previous industrial development in Europe. Experience proved that American conditions, climatic, industrial, social and economic, differ so seriously from the European that these attempts were not very successful. The casein plastics industry then entered a period of readjustment, adapting itself and its product to fit better into the American industrial plan.

Progress has necessarily been slow, but considering the adverse economic conditions of the past eight years, it is quite hopeful. Only the button industry has been materially affected, and progress has been made more in the business than in the scientific and technical fields. The next step obviously is more scientific research in order to adapt the material to application on a broader industrial basis, and to improve further the technic of manufacture to adapt it to the tempo of American mass production. There is evidence that a definite trend in this direction has been followed for some years. There is also evidence that work has been started to pursue this trend more intensively, not only in order to broaden the possible industrial field for casein plastic material, but to include other protein materials. If this work is successful, protein plastic material may be expected to fill an important place among our medium-priced to cheap plastics.

History of Development

Among the early records of work done to produce a plastic material from casein is a German patent issued in 1885 to E. E. Childs of Brooklyn, N. Y.,[77] and a United States patent in 1886 to Mestaniz.[256] In neither case is any hardening agent mentioned and the products,

achieved after rather laborious and crude processes, could have had no possible industrial application. There is no record of any having been made.

According to Karl Wernicke[384] and Eugen Stich,[357] in 1897 there was a demand in German schools for a white "blackboard." The publishing and lithographing company of Eiler and Krische undertook the problem and tried, among other things, a casein coating on cardboard, but the results were not satisfactory. Krische secured Adolf Spitteler, a chemist, to work on the problem. In experimenting further with casein coatings, Spitteler found that formaldehyde produced with casein a tough, insoluble, horn-like mass. The importance of this was recognized by both Spitteler and Krische and patents covering the fundamentals were taken out, the most important being German patent 127,942.[231]

Two years of work on development demonstrated the fact that a large capital and a staff of technical men would be required to achieve success. Accordingly they induced the Vereinigten Gummiwarenfabriken of Harburg and Vienna to take up the problem. In 1900 they sold the French rights to August Pellerin and Leon Orosdi and established for their product the trade name "Galalith" from the Greek, meaning "milk stone." In 1904 the French and German interests merged to form the Internationale Galalith Gesellschaft Hoff & Co. This company, the pioneer in the industry and the only successful manufacturer of casein plastic material in the world until the lapse of the basic patents and the World War made it possible for others to follow, is still one of the largest, if not the very largest producer in the world. So well have they established their product that "galalith" has become a common descriptive term applied by the trade to casein plastics in general, without regard to source, just as Celluloid became celluloid and Linoleum linoleum in other industries. According to Wernicke,[384] in 1912 the Internationale Galalith Gesellschaft at Harburg employed 350 persons in a plant having a total floor space of 21,000 square meters and containing more than 350 machines, most of them electrically driven. Within the past few years they have established a branch in London, Galalith Ltd.

According to the Chief Chemist and Works Manager of Erinoid Ltd., Robert Dodd,[112] in 1909 Victor Schultze, a Russian student, patented a process for the manufacture of a solid plastic from milk curd.[327] An English concern bought the patent rights and spent several years and much money in unsuccessful attempts to produce a salable product. About the time of the World War, or shortly after, some Irish capitalists were interested in the project, which was finally successful. The product was given the trade name Erinoid.[11, 113] For many years it

has been the most widely used casein plastic manufactured in Great Britain.

With the expiration of the basic patents, companies were formed all over the world to manufacture casein plastic material. For various reasons, many of these enterprises were unsuccessful but a substantial number survived and are still in business. A partial list of their trade names, taken with corrections from *Chemical & Metallurgical Engineering*, Directory of Plastics, November 1934, is as follows:

Adarola	Jose Oregui, Renteria, Spain
Akalit	Akalit Kunsthorn-Werke A.-G., Vienna
Aladdinite	Aladdinite Co., Inc., Newark, N. J.
Alkalith	Alkalith Kunsthorn-Werke A.-G., Vienna
Ambloid	Dai Nippon Celluloid Co., Ltd., Osaka, Japan
Ameroid	American Plastics Corp., Bainbridge, N. Y.
Argolite	Chemische und Kunsthornwerke, Perov, Czechoslovakia
Axolith	Soc. l'Oyonnaxienne, Oyonnax, France
Beroliet	Italy
Caseilithe	Soc. Industrielle du Celluloide, Paris, France
Casolith	Hollandische Casolith Werke, Holland
Corozite	Soc. anon. Prodotti Italiana Corozite, Gorlago, Italy
Dorcasine	Chas. Horner, Ltd., Halifax, England
Erinoid	Erinoid, Ltd., Stroud, Gloucester, England
Esbrilith	Rheinisches Farbwerke, Berlin, Germany
Gala	Cie du Celluloide-Petit-Collin-Oyonnithe, Paris, France
Galakerite	Soc. Ital. Galakerite, Milan, Italy
Galalith	Internatl. Galalith Gesellschaft Hoff & Co., Harburg-Wilhelmsberg, Germany
Galapol (Dip polish)	
Galoid	
Globerite	Soc. Ind. Globerite, Milan, Italy
Glorit	Kunsthornwerke Schiel & Co., Sternberg, Czechoslovakia
Hastra	Hastra Kunsthorn-Presswerke, Vienna
Hornit	Gottfried Probst, Nurnberg, Germany
Ideallith	J. N. Bolkurt, Nurnberg, Germany
Ivogallith	France
Ivryne	Estab. A. Feuillant & Fils, France
Keronyx	Aberdeen Combworks Co., Ltd., Aberdeen, Scotland
Lactilith	France
Lactite	
Lactitis	
Lactoid	British Xylonite Co., Ltd., Hale End, London, England
Lactoite	
Lactolithe	Soc. Nobel Francaise, Paris, France
Lactoloid	Dai Nippon Celluloid Co., Ltd., Osaka, Japan
Lactonite	Erste Estnische Kunsthornfabrik, Fallin, Esthonia
Lactonith	
Lupinit	Lupinit G.m.b.H., Manheim, Germany
Luxalith	
Luxolith	Soc. l'Oyonnaxienne, Oyonnax, France
Moroid	Morgan Ebonite Co., Ltd., Manchester, England
Moskalit	Moscow State Chem. Trust, Moskva, Russia
Neolith	Deutsche Kunsthorn Gesellschaft, Germany

Omnilithe	Motende, Charente, France
Osalith	M. Oechsler, Ausback, Germany
Oxygalalith	France
Oyogalithe	Soc. l'Oyonnaxienne, Oyonnax, France
Oyonnithe	Soc. Petit-Collin-Oyonnithe, Paris, France
Paragalithe	O. Rabault, Paris, France
Proteolite	Industria Italiana della Proteolite, Milan, Italy
Rexalith	Soc. la Rexalith, Paris, France
Similithe	Lagnionie et Cie, Paris, France
Syrolit	Aktiebolaget Syrolit, Eslof, Sweden
Verolith	Verolith V.m.b.H., Nurnberg, Germany
Zoolite	Soc. Polenghi Lombardo, Codogno, Italy

It is reliably reported[302] that, in 1931, 20 out of 50 German plastics manufacturers failed because of economic conditions. As the above list does not contain anything like that number of German-made casein plastics, it is obviously far from complete, so that only deductions of a very general nature can be drawn from it. One fact, however, stands out clearly. Casein plastic material is much more broadly applied and more important as a material and as an industry in Europe than in the United States. According to Dodd[114] casein plastic material finds application in 30 to 40 trades in Europe.

The European manufacturers of casein plastic material have for some years recognized the value of trade associations. In Great Britain[373] they have been very active and have done good work, both in controlling the quality of the product and trade practices, and also along educational and social lines among the members. The trade associations are also strong in Czechoslovakia.[397] In Italy[91] they are fairly active and strong but under government control, while in Germany[302] they have almost entirely ceased to operate for the industry in general, but have reorganized along specialized trade lines, such as button manufacturers, etc. In France the movement never gained much foothold.

The first successful casein plastic material to be developed in the United States was produced by Christensen about 1919 and was sold under the trade name Aladdinite. In 1924 Karolith was produced, followed by Kyloid, Inda, and Erinoid. These were all successful products insofar as they were all good casein plastics that compared favorably with the European products. During these years there were also numerous unsuccessful attempts at development, which were largely promotional schemes in which an unscrupulous exploiter sold a gullible investor the idea that he possessed the very secret formulas of the process and could develop galalith if enough money were advanced. The successful processes were, without exception, the result of American development from American research or of the purchase of European processes from reputable concerns.

Although successful as far as the production of the material was con-

cerned, the industry was not so successful in the application of the material to American industry. Many European applications were found to be impracticable because of differences in climatic conditions. The process of manufacture was long and costly and the material could not be worked advantageously in speedy automatic machines. Added to this was the fact that, since the scrap could not be reworked and there was no profitable outlet for it, it was entirely waste, amounting in some cases to 50 per cent or more. This naturally made competition with established materials extremely difficult. The industry, the material, or both, had to be readjusted to fit into the American industrial scheme or be content with a very small business.

This readjustment started in 1928 when George Morrell, a button manufacturer, took over the struggling Kyloid plant and prepared to make finished buttons from casein direct. He was followed by Christensen, who in 1929 organized the Button Corporation of America to fabricate Aladdinite directly into buttons. As a casein plastic raw material, Aladdinite is now practically unavailable. Other concerns that joined the casein plastic plant directly to the button or buckle factory are Synthetic Plastics Co. of Newark, N. J., Rochester Button Co. of Akron, N. Y., The Voges Manufacturing Co., Ozone Park, N. Y., and Valenti Horn Button Works of Astoria, L. I., N. Y. The volume of business done by these companies is comparatively large, something over 8,000,000 gross of buttons annually, and it promises to increase, as this union has materially reduced costs and eliminated waste, enabling the casein plastics button to compete successfully in the lower price field.

In 1931 the Karolith Corporation, Erinoid Company of America, American Machine and Foundry Company, and Panplastics Corporation merged their casein plastics units to form the American Plastics Corporation. The product was given the trade name Ameroid. It is today practically the only American casein plastic raw material available to the trade as such, and the major part of it is sold in the form of button blanks rather than as sheets and rods.

This brief outline of the development of casein plastic material may be inadequate for some readers. Recent European development has not been considered, owing to the fact that little of it is pertinent to the development of the present chapter and also because it has been thoroughly covered by other writers.[55, 92, 106, 107, 245, 276, 293, 311, 312, 352]

Manufacture by the Wet Process

Two general methods have been used to produce casein plastic material; these are known as the wet and the dry processes. They are both built around the fact that casein masses are rendered tough and comparatively insoluble by the action of a hardening or tanning agent.

They differ fundamentally in the fact that in the wet process the casein is dispersed in aqueous alkaline solutions, while no such step is involved in the dry process.[395] Acid caseins were used in the wet process because they are more readily dispersible in aqueous alkaline solutions. Since it has been found that in general rennet casein produces a better product in the dry process,[50, 141] this type is commonly used in the industry at the present time.[114] The dry process is the newer and improved method.

The basic patents of Krische and Spitteler[231] specified the dispersion of casein in an alkaline solution, its coagulation with acid, and the subsequent treatment of the pressed mass with formaldehyde. Thus the industry was started with the wet process of manufacture and it followed this course through its early years. The process of Victor Schultze,[327] which was probably the first in Great Britain, was also a wet method and it was largely for this reason that it proved unsuccessful. Wolter[396] describes such a process in detail, but his description indicates that he did not himself have first-hand experience because many of the details are ambiguous. This fault is rather common throughout a large proportion of the early literature on the subject, particularly the patent literature.

Since the wet process is of interest only historically and academically, a brief outline of it will suffice for the present purpose. Acid casein dispersed in an aqueous alkaline solution, and treated with dyes, fillers, etc., as desired, is coagulated with an acid or an acid salt. The curd is pressed at moderate pressure to remove the major part of the water, then in a hydraulic press at approximately 500 pounds per square inch to convert the curd to a rubbery, translucent (if fillers are not added) plastic. This may take from one to 24 hours, depending upon the thickness of the sheet desired and upon the temperature of the press. The soft plastic sheet is placed in a bath of aqueous formaldehyde to harden, where it must remain for three weeks to three months, depending upon the thickness of the sheet. When thoroughly hardened, the material is allowed to dry or season, and it is then ready to be used for fabricating buttons, beads, etc.

Two modifications are of interest because they eliminate the hardening treatment. In one[48, 208] the casein is dispersed in aqueous ammonia, and formaldehyde solution is used as the coagulant. In the other[194] casein suspended in water is treated with acetaldehyde and an organic acid. It is then coagulated with an alkali. In both cases the pressed coagulum hardens without the long treatment in the formaldehyde bath, and requires only seasoning to be ready for application. It has been found, however, that wet-process material is usually more brittle[295] than the product of the dry process. Patents covering wet processes of manufacture are listed among the references at the end of this chapter.

This is by no means a complete list but is representative and from it may be gained a fair idea of the process.[29, 38, 126, 170, 215, 231, 235, 244, 255, 256, 291, 294, 339, 348, 356, 370, 399, 400]

Manufacture by the Dry Process

Rennet casein is used almost universally in the dry process of manufacture of casein plastics.[144] A description of the process of making rennet casein is given in Chapter 4 but a brief outline from the point of view of the plastics industry is desirable here.

It is essential that only pure, sweet, practically fat-free skim milk be used in the manufacture of rennet casein for the plastics industry.[243, 300] The process is somewhat more involved and requires closer check than that for the production of acid caseins because the chemical reactions involved are much more complicated.[39] The product should be granular, of light straw color, practically tasteless and with a very faint, pleasant odor. The pH should be maintained close to 7.0,[93] the moisture content between 7 and 10 per cent, and the ash content 7.5 per cent.[58] A lower content of ash indicates mixture with free casein, an undesirable feature resulting from the use of partially sour skim milk;[136] a higher content of ash indicates the presence of inorganic impurities. It is a comparatively simple matter to check rennet casein by analysis[104] and to test for microörganisms[78] before it goes into the process of manufacturing plastics, and care in this regard is likely to pay well in greater uniformity of product and process. According to Simmons,[333] the best rennet casein for the manufacture of casein plastic material is produced in France, especially in the Department of Charente-Inferieure. In the United States at the present time there are three principal producers: Casein Manufacturing Company, New York City, Dairy Products, Inc., Chicago, and the National Casein Company, Chicago.

Unless ground at the casein factory, a customary procedure in the United States, the casein is supplied in the granular form in which it comes from the drier. Before mixing, it must be ground to pass a 40-mesh sieve, with but a very small percentage of very fine powder of 100-mesh or finer. Two forms of grinders or pulverizers find rather general use for this purpose, mills with series of corrugated rolls, such as are used in flour mills, and impact pulverizers. Porcelain-lined ball or pebble mills are not to be recommended because material ground in them is apt to contain grit unless very special care is exercised. The presence of a small proportion of grit renders the casein fit only for the production of inferior material, as it prevents attaining a satisfactory polish on finished articles. If carelessly handled the casein may have pieces of wood, brick, stone, etc., mixed with it. These must be removed carefully before grinding, a tedious and somewhat costly operation. It is therefore well to investigate the source of supply before

buying and to secure supplies of casein only through reliable concerns. As a further precaution and as a check on the grinding operation, it is advisable to sift the casein before mixing.

Mixing and Moistening

Satisfactory results may be obtained with any type of mixing machine that thoroughly turns the powder over and over on itself, that has no



Courtesy of the Artofex Corporation

Figure 25.—Dough mixer extensively used in plastics industry

dead spaces where some of the powder can lie undisturbed, and that does not beat the mixture and thus produce excessive dust from the dry or nearly dry powder on which the whole operation is performed. Blücher[52] gives a good general treatment of dough mixers which may be used. The Artofex mixer shown in Figure 25 is employed extensively both in the United States and in Europe by casein plastics manu-

facturers. It is efficient, simple to operate and keep clean and, with its removable containers on wheels, it renders the handling of the finished mix both convenient and economical. Alfred Kohner[222] has patented a device for blowing water in the form of steam or fine spray into the casein powder. Julius Kathe[216] also has patented a process for treating ground casein with water vapor. It has also been proposed[340] to add water in the form of crystals containing water of crystallization, glucose syrup, glycerol, etc. As modifications they are of interest, but unnecessary with a good mixer.

The percentage of water added may vary within rather wide limits and depends largely upon how the plastic is to be worked. The greater the proportion of water the softer the plastic and the greater the shrinkage on finishing. Boerma[54] and Amandus Bartels[30] specify 29 to 42 per cent, the Deutsche Kunsthorn Ges.[110] 45 per cent or above, and Simmons,[333] taking into account the moisture content of the casein, suggests the addition of water to total 40 per cent. Von Koch[220] proposes an alcohol treatment in order to obtain uniform distribution of moisture throughout the individual granules of casein.

If the material is to be colored, acid dyes are dissolved in the water before adding it to the casein powder. To obtain an opaque material, a white pigment, such as lithopone, titanium dioxide, titanium-barium pigment, etc., is first mixed dry with the casein. If white material is to be produced, somewhat more pigment is used than for opaque colors and only clear water is added. Colored pigments or lakes may be used in place of the pigment-dye combination, but the cost is higher and the results not so pleasing. For translucent and transparent effects the pigment is of course omitted. For metallic effects, such as gold, silver, etc., bronze powder is added in place of white pigment. A number of modifications will be found suggesting the addition of volatile organic substances, such as alcohol, acetone, ethyl lactate;[320] neutral phosphate esters or reducing agents;[269] carbonates, bisulfides, etc.,[190] to render the material porous; talc, steatite, benzanilide, etc.,[205] to produce a pearl effect. Any such modifier should be added with the water before mixing.

Addition during mixing of numerous substances intended to act as plasticizers to make the product less brittle has been recommended. Among the proposed plasticizers are glycerol,[31, 295] aniline oil or acetanilid,[281] sulfonated castor oil,[383] ethereal alcohols having at least two groups substituted by oxyalkyl groups,[324] higher alcohols from dodecyl to octadecyl alcohol,[260] acetic acid,[305] 20-per cent ammonia,[249] acetone-glycerol benzoate in butyl alcohol,[108] the oleic ester of hydroxyethane sulfonic acid,[109] triethanolamine, other ethanolamines or ethylene diamines,[101] amines of the fatty or of the aromatic series,[253] methylol ethyl urethane,[200] and cyclohexylethanolamine.[323] These and many

more are suggested to plasticize and soften the casein.[103] It is questionable whether in the present method of manufacturing casein plastics these substances accomplish their objective. Water alone plasticizes the powder adequately and the long soaking in the hardening bath tends to remove the added substances, which are soluble in water, or so to modify their action that it is doubtful whether they are beneficial to the finished product. The proposal, however, is interesting evidence of the recognition of a weakness in casein plastics and of attempts to correct it.

According to Bartels,[377] "The finely powdered casein mass quickly soaks in the water and is still powdery after mixing." During the mixing, appreciable heat is generated in the powder, the so-called heat of absorption. If the mixing is not properly done (either from the use of the wrong type of mixer or from interruption of the process before its completion) and if the soft lumps formed by the first contact of the water with the casein are not broken up promptly, this heat causes partial plasticization, with the result that the lumps become very tough. In order to restore homogeneity to the mixture, it is then necessary to sift out the lumps, reduce them to powder, and mix it well with the rest of the batch.

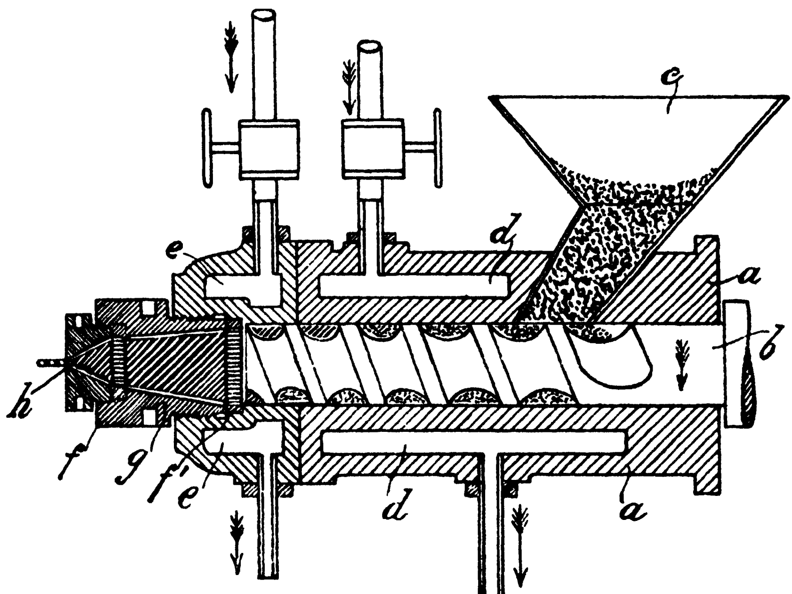
The dry powder, like the original dry granulated casein, keeps well in storage and, if protected from moisture, rodents, and insects, will keep almost indefinitely. After mixing and moistening, however, the powder should be used promptly or putrefaction will set in. Simmons[333] suggests that the batch be allowed to stand at least an hour after mixing to permit diffusion of the water into the casein particles, while an anonymous writer[6] says that it must be used within 10 or 12 hours after mixing or it will become unfit for use. This time can, of course, be extended somewhat by keeping the powder in a cool place, but after it has begun to spoil it is difficult to work and the quality of the resulting product is inferior.

Extrusion in Form of Rods

The conversion of the powder to the soft plastic requires the application of pressure at elevated temperature.[254] In order to insure strength in the plastic[28] there must also be mechanical working in such a way as to force the material to flow "onto and almost into itself" by shearing, smearing, or some similar action involving flow throughout the mass. Various devices are employed in the trade to accomplish this result,[146] among them the heated cylinder press with rotating screw,[6] as used in the rubber industry, steam-heated kneading machines,[30, 52, 85, 290] and rolls with considerably less differential than when used for milling rubber.[110, 111, 202] Mention should also be made of machines that mix

and plasticize the material at the same time, such as the Bridge-Banbury kneader[102] and others,[298] also of mixing-extrusion units[248] and other types of extruders that might find application.[178, 199]

The type of machine that finds general application among American casein plastic manufacturers is the heated cylinder press with the rotating screw. Patents covering the application of such a machine to the casein plastics industry were taken out by the Internationale Galalith



Courtesy of The Chemical Foundation, Inc., Owner

Figure 26.—Diagram of screw-press extruder. Reproduced from U. S. Pat. 1,211,526.[33]

Gesellschaft Hoff and Company,[201] Amandus Bartels,[33] and E. H. Johnson.[211] A diagrammatic sectional drawing through the cylinder of such a machine is reproduced in Figure 26. It shows that the machine consists essentially of a heavy conveyor screw or worm (*b*), operating through a cylinder that is jacketed for water circulation (*d, d*), and partially through a head that joins on and is really a continuation of the cylinder, jacketed for circulation of steam (*e, e*). A cone-shaped bushing (*g*) screws into the head, while the mouthpiece or nozzle (*h*) with bore the size of the rod required, screws into the bushing. A sieve or breaker (*f'*) fits in between the head and the bushing chambers and a smaller breaker (*f*) between the bushing chamber and the nozzle. These breakers are steel discs, *f'* about ½ inch thick and *f* about ¼

inch thick, and perforated with holes from approximately 3/8 inch to 1/4 inch in diameter.

The powder mixture is fed into the cylinder from the hopper (*c*), sometimes by hand but more frequently by mechanical semiautomatic devices. It is important that the feed be uniform in order to produce rods of uniform diameter and smooth surface, thus reducing loss in grinding them to size. It is important for the smooth operation of the machine that in addition to the steam-heated head, the nozzle be heated[169, 333] either by means of an electric unit or by a small gas flame.

After the powder mixture enters the cylinder, where it is kept cool by cold water circulated through the jacket, it is conveyed by the screw to the heated head. Here it piles up against the first breaker, thus generating pressure, until, through the combined effects of heat and pressure, it is converted into a soft plastic condition and is forced through the holes of the breaker. It continues to be heated while it is forced through the holes of the second breaker and out through the nozzle, issuing in the form of a rod of soft, hot plastic. If rods are desired as the finished product, this plastic rod is cut off in the desired length (usually one meter), placed immediately in cold water where it is left until thoroughly cold, and then transferred to the hardening tank. If sheets are to be produced, the plastic rod is cut into short lengths which are piled together and wrapped in a clean cloth until ready to be laid out for introduction into the hydraulic press. Not only is the powder mixture fed to the machine more slowly and uniformly when rods are being produced, but frequently sufficient heat is built up by the friction of the plastic to render steam heat on the head undesirable. Excessively hot plastic may produce not only irregularly surfaced or bumpy rods, but also oversized rods and sometimes countless tiny bubbles (pinholes) throughout the rod. This latter phenomenon is apt to occur if the rods are not promptly placed in cold water after being cut off. On the other hand, these considerations are unimportant in producing sheets because the subsequent pressing smooths out irregularities. In order to obtain maximum production the powder is fed as rapidly as the machine will safely take it and the heat is maintained as close to 180°F.[333] as possible.

There are a number of modifications of the machine or method just described, among which may be mentioned a nozzle attachment[32] for control of the size of rods extruded, an attachment to the nozzle to form the plastic into blocks under pressure,[30] extrusion of the plastic into a sectional mold,[288] a hydraulic attachment and accumulator to take care of the back pressure on the screw and hold the pressure on the plastic constant,[25] a gauge that may be placed in this hydraulic line to record the pressure,[246] the screw bored to permit circulation of water through it,[213] a threaded screw ejector to permit removal of the screw even

when the machine is full,[369] production of multicolored effects by having three screws deliver plastic independently into a single chamber from which it is extruded by a fourth screw,[122] production of multicolored effects by having the screw shaft bored to admit a second smaller screw to operate with both screws discharging through a common head,[66] modification of the nozzle for the extrusion of tubes,[8] and a head carrying three sets of breakers instead of two.

The heated cylinder press with rotating screw is an excellent machine for the continuous production of rods and tubes, the latter requiring a nozzle fitted with a mandril, and slower more uniform feeding of the powder. It is possible to extrude the soft casein plastic from a hydraulic stuffer, such as is used in the celluloid industry, but this machine has two disadvantages. In the first place the operation is discontinuous instead of continuous; it is necessary to fill the cylinder with a charge, extrude, and then refill, the extrusion necessarily being interrupted during refilling. In the second place the powder is merely plasticized and the plastic extruded, whereas the conveyor screw rubs and smears the plastic into itself while exerting pressure upon it. In order to produce a strong material, pieces of the soft plastic rather than the powder should be loaded into the stuffer, thus entailing an additional and unnecessary step in the process.

Formation of Sheets from Soft Plastic

Sheets are prepared from the soft plastic rather than from the powder. One of two methods may be used: First, by pressing the soft plastic material in hydraulic hot presses until it fills frames of the desired size and thickness and, second, by slicing sheets of the required thickness from solid blocks of the soft plastic of the required size. This size may and does vary somewhat, but the major part of the sheets produced is in the approximate size of 16 by 20 inches (40 by 50 centimeters), a size established by the Internationale Galalith Gesellschaft years ago and to which the trade has become accustomed.

The frames used in the first method are about 10 per cent larger in all dimensions than the size desired for the finished sheet (to take care of shrinkage during drying) and are heavy enough to withstand the pressure exerted upon them in the press. The charge of soft plastic (rods from the extruders, strips from rolls or pieces from kneading machines), is weighed to just fill the frame and leave a slight excess to form "fins," and placed on a thin metal sheet (polished zinc serves very well) together with and surrounded by the frame. It is essential for the production of material of high quality that this weighing be reasonably accurate. If the material is underweight the sheet will not be completely filled out and hence imperfect; if it is overweight the

excessively heavy fin will indicate that the sheet is too thick and probably uneven in thickness. A second thin metal sheet is placed over the frame and its charge and the whole assembly placed between heated platens in a hydraulic press capable of exerting a pressure of approximately one ton per square inch of material.

Regular standard multiple platen presses having from six to twenty openings and platens constructed to permit heating with steam and

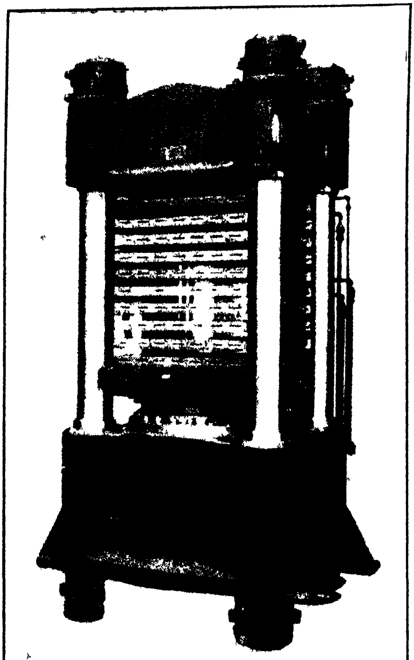


Figure 27 — Multiple-platen hydraulic sheet press

Courtesy of Chas. F. Elmes Engineering Works

cooling with water have been found satisfactory. Figure 27 shows such a press. Modifications, however, are of interest.[45, 279] It is advantageous to have the production presses operated by low and high pressure accumulators, low pressure for closing, high pressure for forming the sheets. When all the openings in the press have been filled as described the press is slowly closed and full pressure slowly and evenly applied. After holding full pressure and heat for a certain time, which varies with the thickness of sheets being pressed, the steam is turned off and cold water circulated through the platens without releasing the pressure. When completely cold, the press is opened, the sheets removed, the metal sheets stripped off and the sheets of soft plastic knocked out of the frames. The smoother the surface of the metal sheets the smoother

is the surface of the soft plastic. After trimming off the fins the sheets of plastic are ready to be placed in the hardening bath.

The second method, namely slicing sheets from a solid block of the soft plastic, is not in as general use as the first method, but it has a great advantage over it in the fact that by the slicing method it is possible to produce sheets of absolutely uniform thickness. Much experimentation[9] has been done in an effort to make this method practicable for production, but to date the quantity of material produced in this way is not large.

The veined or streaked effects, such as the various horns, tortoise shell, jade, etc., are obtained by introducing small pieces of the soft plastic in the color of streak desired[263] into the powder before it is fed into the extruder or run through the rolls. These may be made in a number of ways, one of the simplest being to mix and extrude the plastic in the usual manner except that, in place of the customary nozzle, one having six to eight openings is used, each opening extruding a rod ⅛ inch or less in diameter. These rods are cut off in any convenient length and cut into lengths of about ½ inch, more or less as desired, by means of a shearing machine of some sort. If it is desired to produce long streaks of color, these "buttons" must be used comparatively soon after extrusion, or be kept moist. If short, blotchy streaks such as those in tortoise shell are desired, the buttons are allowed to air-dry for some hours before mixing. The best effects are produced when rods of about ¼ inch diameter are used, rather than the ½ inch usually used for the production of solid color sheets. In extruding them the mixture of powder and buttons is fed to the machine as fast as it will take it, the machine being maintained hot, the breakers omitted, and a two-opening nozzle used. The rods, gnarled, bumpy and very uneven of surface, are cut into short lengths, six to eight inches, and when loaded into the frame are laid in the very center with good space between the rods and the edge of the frame in all directions. This, of course, is to secure maximum flow, thus drawing out the streaks even more than was done in the rods.

In the production of streaked rods, the buttons of soft plastic should be quite fresh or soft. The extruder is run more slowly, that is, the feed is more like that for solid-color rods, the operating temperature is the same as for regular rods, the nozzle is of single opening, the size of rod desired, and usually one or both breakers are omitted because they tend to churn and mix the plastic too much. Effort is made to reduce the irregular, bumpy surface as much as possible because this represents loss. It is necessary to grind the rods down to a smooth surface to render them salable. This loss must be expected and allowed

for, however, since streaked rods when extruded always have a rougher surface than rods of solid color.

Attempts to develop methods or treatments for the production of transparent material date from Spitteler's initial work in 1899. Spitteler's method[348] consisted in treating an alkaline dispersion of casein with an excess of caustic. Solid matter settles out on standing, and plastic material made from the casein in the decanted clear dispersion is transparent. Reithoffer[306] treated skim milk with soda ash, centrifuged out the solid impurities, and made transparent material from the casein coagulated from the purified milk. Bartels[34] prepares his casein for the production of transparent material by rubbing the curd to destroy the "layer structure." He also[35] extracts powdered casein with alcohol, benzene, or both to remove the last trace of fat. Landecker[237] does the same thing with methyl alcohol, ethyl alcohol, or mixtures of the two. Carpender[67] washes a hydrochloric acid and water suspension of casein with strong solutions of magnesium sulfate to remove cloudy impurities. Alden[4] mixes oxycellulose and ammonia with casein, Heckel[173] mineral oil sulfonates, and Dumont and Lück[117] use mixtures such as ethyl-benzylanilin and dichlorohydrin with casein to produce a transparent material. Kratz and Heckel[230] have a special apparatus for the production of transparent sheets. The confusion so manifest in these divergent efforts seems to emphasize the necessity for a more scientific approach to the problem.

Hardening the Soft Plastic

The hardening of the soft plastic is the most important, the most critical, the most disagreeable, and the most expensive step in the process of manufacture of casein plastic material. The tendency for some years has been to shorten it and to attempt the correction of some of its worst features while retaining the all-important reaction between casein and formaldehyde. It has been suggested that hardening accelerators, such as alkali or ammonium salts[203] or alkaline or alkaline earth thiocyanates[206] be mixed with the powder before extrusion. Other developments are noted farther on.

The hardening process is essentially a reaction between casein and formaldehyde. It is necessary, therefore, that the formaldehyde solution penetrate the casein mass completely. The thickness of the mass governs the time of hardening, which may vary in practice from about one week to six months. It is also dependent upon the concentration of formaldehyde in the bath, the temperature at which it is maintained, and the pH. There is considerable disagreement in the literature about the concentration of formaldehyde to be maintained in the bath. Clement and Rivière[85] recommend 35 to 45 per cent, Hemming[175] 42 per cent, Stich[358] not over 35 per cent, Blücher[52] maintains that there are no

reliable statements, Haupt[169] specifies 3 to 7 per cent, Mougeot[265] 6 to 10 per cent, and Dodd[114] 4 to 5 per cent. The last three are approximately correct because it is known that the usual concentration in the baths is 4 to 5 per cent and that it is important that this concentration be carefully maintained. The pH should be watched and held within the limits of 4.0 to 7.0.[192, 265] The temperature should be regulated closely at 60°F. according to Simmons,[333] for if it falls to 50°F. or below for any length of time the reaction practically ceases. In the United States it is the usual procedure to maintain the temperature of the bath nearer 75°F. The room containing the formaldehyde tanks should be well ventilated and the workmen equipped with good rubber gloves. The vapor from even a 4 per cent solution is not only very irritating to the nasal passages and the eyes but it has decided physiological reactions upon those too frequently exposed, taking the form of dermatitis, throat irritation or nerve exhaustion. It will also react with any casein in process of manufacture or in storage, rendering it unfit for use in casein plastics manufacture.

For introduction into the formaldehyde tanks, the sheets, rods, and tubes are set in suitable frames that are designed not only to hold the material properly but to allow free circulation of the formaldehyde solution on all sides. This should be mechanically circulated at least twice a day by means of a pump, drawing from the bottom of the tanks and discharging at the top. It should also be frequently analyzed, tested for pH, and be thermostatically controlled. The material should be examined occasionally for penetration of formaldehyde as a check and also to determine definitely when it is ready for removal. A rough test may be made by placing the piece to be tested in boiling water for a few minutes. Lack of homogeneity in a partially hardened piece is quite apparent. A more accurate method is the examination of a section under a microscope with polarized light.[172, 275]

Attempts have been made to substitute other agents or methods for the formaldehyde bath. Formaldehyde vapor[358, 368] has been suggested, as has the polymerization product of formaldehyde with sulfuric or formic acid,[319] chromium salts and tannic acid,[290, 391] hydrogen peroxide[401] and alum.[175] Alum is used also to tan glue and gelatin.[286, 341] With the exception of alum, which is applied in a different connection discussed farther on, none of these hardening agents has proved commercially important up to the present time.

Seasoning

Upon completion of the hardening the material must be seasoned, or dried. This also is a slow process, taking about the same length of time as hardening, because excess water and formaldehyde must be expelled without rupturing the material or setting up stresses and strains within

it. It can be safely speeded up somewhat by drying at a moderately elevated temperature in a carefully controlled humidifier, keeping the surface layer soft so that the moisture in the center can readily diffuse out. Elevated temperatures without humidification are not recommended. For reasons already given, precautions must be taken to insure good ventilation.

During drying or seasoning the material usually warps more or less. This is especially true of the sheets, which must be straightened before they can be stored or sold satisfactorily. Straightening is accomplished in low-pressure hydraulic presses where the sheets are slowly heated between platens heated by steam until the sheets are flexible. The press is then closed and cold water is circulated through the platens until the sheets are cold. It is sometimes found expedient to employ two presses side by side, one hot and the other cold. As soon as the sheets in the hot press are heated enough for the press to be closed the hot sheets are transferred to the cold press to cool under pressure. When the sheets are removed cold they are quite flat and smooth. If properly hardened and seasoned they remain in this condition until climatic conditions or external pressure bends them out of shape. If carefully handled through seasoning, rods, except those with small diameters, seldom require straightening. If as usual a very uniform or exact diameter is required or if the surface is rough, as is usually the case with mottled rods or those carelessly extruded, the rods must be ground or "trapped" through a suitable machine. Sheets are sometimes sanded to the required uniform thickness. Since both operations are very wasteful, it pays to process the material in such a way as to insure maximum uniformity.

In general this is the process used for the manufacture of casein plastic material into sheets and rods. There are naturally many modifications, such as the forming of two sheets of soft plastic into one by moistening contacting surfaces with lime water,[36] an alkaline casein dispersion,[317] or thin sheets of celluloid[282] and repressing, both hot and cold. There are a number of articles in the literature describing this process of manufacture, among which might be mentioned Stark,[353] Besana,[47] Harry,[167] and especially Simmons[333] who gives in addition a good engineering layout for the process, and Kausch[218] who includes a good patent and literature review.

Cutting or Molding Soft Plastic before Hardening

Casein plastic material in sheets and rods did not fit well into the American system of manufacturing. It is true that it works up well into beautiful buttons and dress ornaments, but it requires a large amount of hand labor and therefore is too costly except for limited application. It was attempted to work the rods into buttons of the

cheaper and larger quantity classes on the Sylbe and Pondorf and other automatic machines[83] but the material was found to be so hard on the cutting edges of the tools that the procedure was impracticable.

In 1929 Christensen found that the incorporation of about 2 per cent of a water-soluble aluminum salt in the soft plastic[80] gave a material rigid enough to be worked in automatic machines (which is not true of the soft plastic made by the customary procedure) and was much easier on the cutting edges of the tools. A special machine was designed[84] to turn buttons varying in diameter from ¼ inch to 2½ inches, in their final form, except for drilling, directly from rods of alum casein. Since the machine is geared to very high speed the production is large.

These buttons are hardened by treatment in a formaldehyde bath, but they are so thin that the penetration takes place in about three to five days. They are then automatically drilled and polished and are ready for shipment in but little over a week. The saving in overhead (capital invested in goods in process) and labor costs is large and there is practically no waste because all turnings and scrap material are alum casein which is readily reworkable. This process enables the casein plastic button to compete with the cheaper, large-quantity vegetable ivory and bone buttons, with the result that in this industry at least, the casein plastic material has an important place. The business has steadily grown and other advances that will be noted indicate what great potential possibilities may lie waiting in the material to be brought out by intensive research.

Recent developments in the button industry in particular and in the casein plastics industry in general have had the same objectives but with somewhat different technics. As has been shown, these objectives are in reality but one, the reduction of the cost of production by reducing the overhead charges and labor costs and by eliminating waste. Attempts have been made to utilize profitably waste galalith as a filler material for other plastics,[329] filler for phenol-formaldehyde molding powder,[124, 177] fertilizer,[271] base for the preparation of lacquer,[116] etc., but to date the best answer appears to be its elimination as much as possible.

The first development was the slicing or cutting of discs or blanks from rods of the soft plastic, or punching these blanks from unhardened sheets. The blanks were hardened in formaldehyde, then turned to shape in machines. The only waste produced was the machine turnings. This is not as efficient as the alum casein method described above, but it does not involve investment in as expensive machinery. The bulk of Ameroid is furnished to the trade in this form.

The next development follows quite logically and is part of the casein plastics tie-up with the button factory. The blanks of soft plastic can be molded to shape if pressed in a button die in a hot mold[262] and the

formed buttons hardened in formaldehyde. Even the holes may be formed in, so that all that is required is the hardening and polishing of the buttons. There is obviously no waste. A modification of this process is to mold the buttons in a multiple die so that the soft plastic in rods, etc., is introduced into the die. A sheet of buttons, connected by a thin film, is formed.[15] The buttons of soft plastic are punched out and hardened in formaldehyde. The fins may be reworked, so here too there is no waste. Attempts have been made to harden the blanks[398] and mold them to shape, but for several reasons this procedure has not proved commercially successful. It has been suggested that in order to accelerate the hardening of the objects they be saturated with ammonium chloride before they are placed in the formaldehyde bath.[207]

The method of molding to shape before hardening is not confined to the button industry, though to date it has made the most use of this development in the United States. To mention but a few possibilities, it has been proposed to mold special rods or tubes[128] by extrusion and to mold articles to form from casein,[49] from thin slices of soft plastic casein,[2] from casein mixed with hot water,[273, 336] from soft plastic molded to shape and surface-hardened,[151] casein molded to shape by water or gas pressure,[278] and hot, soft plastic forced into a cold die to form it.[1, 214]

By molding the soft plastic to shape before hardening, the time required for the process was reduced and the waste material largely or entirely eliminated. The next step would naturally be attempts to reduce further the time required to harden the material and, if possible, eliminate the formaldehyde bath. It was proposed to mold to shape casein with but 7 to 17 per cent moisture content[37] and half-harden with formaldehyde,[337] then to finish the hardening in the formaldehyde bath as usual; also to preform roughly the partially hardened casein, heat, and mold to final form[274] in a cold die.

Other Proposed Hardening Agents and Molding Mixtures

Attempts were made to incorporate in the powder a hardening agent the action of which would either be sufficiently slow to permit molding to form before it would set, or that would require the heat of the molding operation to activate it. Among these may be mentioned trioxymethylene,[261] hexamethylenetetramine,[31, 36] aldehyde-amino polymerization products,[253] diphenol propane and formaldehyde,[200] phenol-formaldehyde,[361] methylol derivatives of urethane,[280] ureas and methylol urethanes,[188] glyoxals,[129, 191, 193] dicyandiamide and formaldehyde,[308] formaldehyde in mixture with a hydroaromatic alcohol or ketone,[359] furfural,[185, 186] mixed aluminates of casein and fatty acids,[388] mixture of casein with alkyl-substituted acrolein,[362] and mixture of casein or soybean protein with formamide and formaldehyde.[98] None of these

has proved of commercial importance, but they are definite indications of a trend of development. The same tendency is shown by attempts to produce a molding composition by treatment with ammonia and boiling,[233] pressing of thin plaques of galalith and horn,[343] benzylation of protein[251] or of casein,[345] and treatment of casein with a tanning agent such as quebracho.[63]

Mention should also be made of the development of plastic molding among the other protein materials. Molding mixtures are claimed for dried blood, water, and filler,[266] bleached with hydrogen peroxide and hardened with formaldehyde,[182] and dried blood, water, and zinc chloride,[183] but it is admitted that this material has serious weaknesses.[171] A development more nearly analogous to that of casein molding mixtures is the case of molding mixtures made with glue or gelatin, which go from a simple mixture of fiber, wax, gypsum, and glue,[316] through mixtures such as glue with methyl alcohol,[307] fillers, fibers, and waxes together with formaldehyde,[115] phenol or α- or β-naphthol and formaldehyde,[160] hexamethylenetetramine,[174] or aluminum acetate,[154] to mixtures of glue, glycerol, and pentosans, heated to generate furfural.[334] Yeast[16, 258] or yeast residues[51] may be mentioned, as well as leather scraps hardened with formaldehyde,[97] with urea, formaldehyde, napthalene, and glycerol,[287] or bark-tanned leather waste;[123] also vegetable proteins[313] such as gluten,[362] zein,[381] cottonseed meal,[350] or soybean meal.[314] The last has found industrial application in the form of a mixed phenol-formaldehyde-protein type of plastic.[74, 365] It is to be noted that the recorded work of Satow on the development of plastics from soybean products was by wet-process methods. His extravagant and often contradictory claims become understandable by comparison with analogous early development of casein plastics. A patent review of these horn-like materials is of interest.[161]

It is interesting to note the development of casein molding mixtures, from those in which casein glue is used as a binding material for inert fillers to mixtures of casein with synthetic resins in which the function of the casein is largely that of filler, probably entering somewhat into the reaction in many cases, but only in a secondary or modifying degree. The patent literature discloses the following mixtures of this kind:

Casein and wood flour[27, 81, 272]
Casein and wood flour treated with formaldehyde[270, 277]
Casein and cork[297]
Casein and cellulose[70, 89]
Casein, lactose, and a tanning agent[266a]
Casein and gelatin[119, 132, 212, 292, 330]
Casein and leather scrap[378]
Casein and slime[69]

Casein and rubber[347]
Casein and vinyl acetate[134]
Casein and cellulose ester[322, 382]
Casein and cellulose acetate[250]
Casein, cellulose acetate, and alkyd resin[59]
Casein and alkyd resin[121, 335]
Casein and alkyd resin treated with formaldehyde[181]
Amino acids in alkyd resins[62]
Casein and phenol-formaldehyde[3, 43, 138, 149, 184, 187, 189, 209, 232, 267, 283, 289, 364, 374, 386]
Casein and urea-formaldehyde[26, 125, 157, 236, 303, 309, 321, 328]
Casein and cellulose[138]
Casein and wood pulp[72]
Casein and portland cement[394]

Analogous molding mixtures with proteins other than casein are also to be found in the patent literature:

Raw hide and urea-formaldehyde[257]
Leather scrap and phenol-formaldehyde[90]
Corn gluten and phenol-formaldehyde[46]
Zein and phenol-formaldehyde[148, 163]
Soybean protein and phenol-formaldehyde[139]
Albumin and phenol-formaldehyde[150, 389]
Glue and phenol-formaldehyde[5, 42, 241]
Vegetable ivory and phenol-formaldehyde[57, 390]

There is clearly evident a trend toward the development of molding compounds from or with protein materials, molding compounds with which articles may be fabricated to come finished from the die. It is well known in the trade that not only is the method of manufacture of casein plastics inefficient and unsatisfactory, but the material produced by it has some serious weaknesses, the worst being its hygroscopicity.[219] This matter will be treated in greater detail farther on. It will suffice to state here that the molded product would tend to be less hygroscopic than the casein plastic material made by the usual methods.

Films from Proteins

Comparatively recently there have been two developments in the industrial use of protein materials that, although not exactly plastic by rigid definition, are yet closely enough associated with plastics to merit brief mention. These are the production of films and the production of synthetic protein textile fibers.

The widespread adoption of the thin transparent films for packaging foods and merchandise sold at retail and the development of safety glass

have created a large demand for film materials. This is largely taken care of by the cellulose esters, but cheaper protein materials mixed with cellulose dispersions[60, 296] or coated with cellulose esters[228] alone or with bonding agents, such as dibutyl phthalate[145] or acetic acid[73] find some application. The great problem with the use of protein films is to insure their flexibility and reduce their hygroscopicity. It is proposed to solve this in the case of gelatin by coagulating with a sulfonic acid from a lime dispersion,[379] mixing with sulfonated castor oil[227] and orthophosphoric acid, mixing with mineral oil[225] and a sulfonated saturated hydrocarbon, mixing with glycols and sulfonated castor oil,[226] mixing with ethyl or benzyl-pyridinium chloride[344] and cellulose, or by hardening with a diketone[351] such as diacetyl, acetyl benzoyl, etc., in the case of zein, or other prolamines, plasticized with dibutyl phthalate and triethanolamine[164] or sulfonamid[166] and hardened with formaldehyde or glyoxal.[165]

A special casein for film production is prepared by coagulating fresh skim milk[86] at pH 4.65 to 4.67, the isoelectric point, thus reducing calcium salts to a minimum and drying to a moisture content of 6.0 per cent or less. Hardened casein films may also be waterproofed by treatment with cellulose lacquer,[224] nitrocellulose, wax and cyclohexyl phthalate,[342] nitrocellulose and chlorinated diphenyl resin.[210] Casein may be dispersed with sulfonated oil,[376] mixed with albumin, caustic potash and glycerol,[332] plasticized with glycerol and sulfonated castor oil and hardened with formaldehyde,[223] benzylated,[155, 252] dispersed with a mixture of ethyl, methyl, propyl alcohols, glycerol, terpineol, and sodium carbonate,[392] coagulated with hexamethylenetetramine, formaldehyde, tannin, etc.[393] There are various ways in which the films can be formed:[230] by feeding powder to the inner face of a band that passes around a molding cylinder,[197] by casting,[315] by casting on a carrier web coated with a film of petroleum jelly,[229] and by extrusion, either adapting some type of extruder designed primarily for cellulose material, [88, 95, 96] possibly with some form of special mouthpiece[315] for the extrusion of very thin sheets, or designing a machine especially for the production of casein or protein films.[360] A casein dispersion may also be used to impregnate a paper web[118] which is then wound on a mandrel to form a tube, and the casein hardened and dried.

Chemistry of Casein Plastics

Any reactions involving protein material are difficult to investigate or to understand. This has always been true and it is still true today, although recently there have been applied to this study such powerful tools as the ultracentrifuge, the x-ray, the infrared ray, monomolecular film measurements, etc. Much experimental data obtained in this way emphasizes the magnitude of the study, for example the determination by

Svedberg and his associates[363] by means of the ultracentrifuge that purified casein is a mixture of micelles of different weights, one of them with a micellar weight of 375,000, while the crystalline protein of tobacco mosaic virus was found by the same method to have a micellar weight between 15,000,000 and 20,000,000.[127]

At approximately the beginning of the present century Emil Fischer built polypeptides from amino acids and broke down proteins by hydrolysis to produce amino acids. He concluded that proteins are long polypeptide chains built up in nature from amino acids. Subsequent investigators have confirmed this conclusion, adding the fact that practically all of the protein "building stones" are alpha amino acids,[387] thirty-one having been isolated to date, seventeen of which are obtained from casein[310] (see also Chapter 2).

Protein material was classed as a colloid until the x-ray showed that there is no such distinct class of substances[19] as differentiated from crystalloids, and that the natural state of any solid matter is crystalline. X-ray measurements show that the protein molecule consists of bundles or masses of long polypeptide chains and is characterized in general by two principal side spacings at right angles to each other,[20] the backbone spacing at approximately 4.5 Ångstroms and the side chains spacing at approximately 10.0 Ångstroms. This is in accord with the theory of the tetrahedral angle of both carbon and nitrogen bonds, and these two make up the backbone of the chain. X-ray measurements also show that the reactive part of the protein molecule is the side chains, and indicates that proteins are built up of filaments (polypeptide chains) linked by primary valences.[18]

Much work is being done in studying the structure and size of the protein molecules by measurements of monomolecular films.[152] There remains much more to be done before very definite conclusions can be drawn but the results in general tend to substantiate the theory of polypeptide chains with side chains and cross linkages.

It is said that polyacrylic acid, and particularly its alkaline salts, give reactions analogous to those of proteins,[355, p. 333] as do also the urea-formaldehyde condensation products.[23] If this is so, careful comparisons of reactions and structure may prove of great value.

The first evidence of a possible chemical reaction or combination in the process of manufacture of casein plastic material is the heat generated when water is added to the powder in the mixer. Although already containing 8 to 10 per cent moisture, the casein absorbs enough water with evolution of heat[217] (unless the first soft lumps are quickly broken up) to be converted into a tough, rubbery form. Hatschek[168] has shown that casein has the power of combining with eleven times its volume of water.

Numerous experiments, principally with fiber proteins such as silk or

wool, have indicated that absorbed water fills in the spaces between the polypeptide chains, since these fibers in steam or water swell much more laterally than longitudinally.[19] X-ray diffraction studies confirm this observation, since after treatment with water the space ring of the backbone remains unchanged while that for the side chains is enlarged.[20]

Interesting data have been obtained on the study of water in casein by means of specific polarization of gels[180] and by the ultrafiltration method.[158] The former method indicates that approximately 6 per cent of water is held in a combined form, between 6 and 21 per cent water fills voids surrounding the gel mass, and that above 21 per cent the structure changes to the open sponge type. The latter method indicates only a small fraction of "bound water" and the authors conclude that an extension of the Langmuir-Harkins theory of molecular orientation would be more in accord with the facts than Kruyt's theory that the stability of the colloidal solution depends upon the degree of hydration (see Chapter 3).

Until recently there was no way to determine how the absorbed water was held and it was impossible to state with any degree of certainty whether this was a true chemical combination. In 1911 Coblentz[87] found that water absorbed by gelatin gave an infrared absorption peak at 3.0 μ. He concluded that there must be some sort of chemical combination. Buswell[65] and his associates recently confirmed these results conclusively, showing that as gelatin is dehydrated infrared absorption at 3.0 μ decreases and is again increased by hydration. As Buswell, Deitz, and Rodebush[64] have shown that 3.0 μ is the band that indicates hydrogen bonding, there is little doubt that the water is held by hydrogen bonds to the gelatin molecule. This work has yet to be extended to other proteins, including casein, but no doubt it will be done soon. Results of such work will probably show that water is held by the different proteins in different amounts, chemically combined by hydrogen bonds.

The conversion of the moist powder into the soft plastic by heat and pressure in the extruder or kneading machine or on the calender rolls is probably the mechanical means of insuring firm bonding. The more completely the material is smeared into itself, the better the probable bonding and the stronger the resulting material. It would be interesting to have, if possible, a series of infrared absorption studies for comparison on the moist powder, the rubbery ball that forms of its own accord, powder merely pressed, and the soft plastic that has been properly worked.

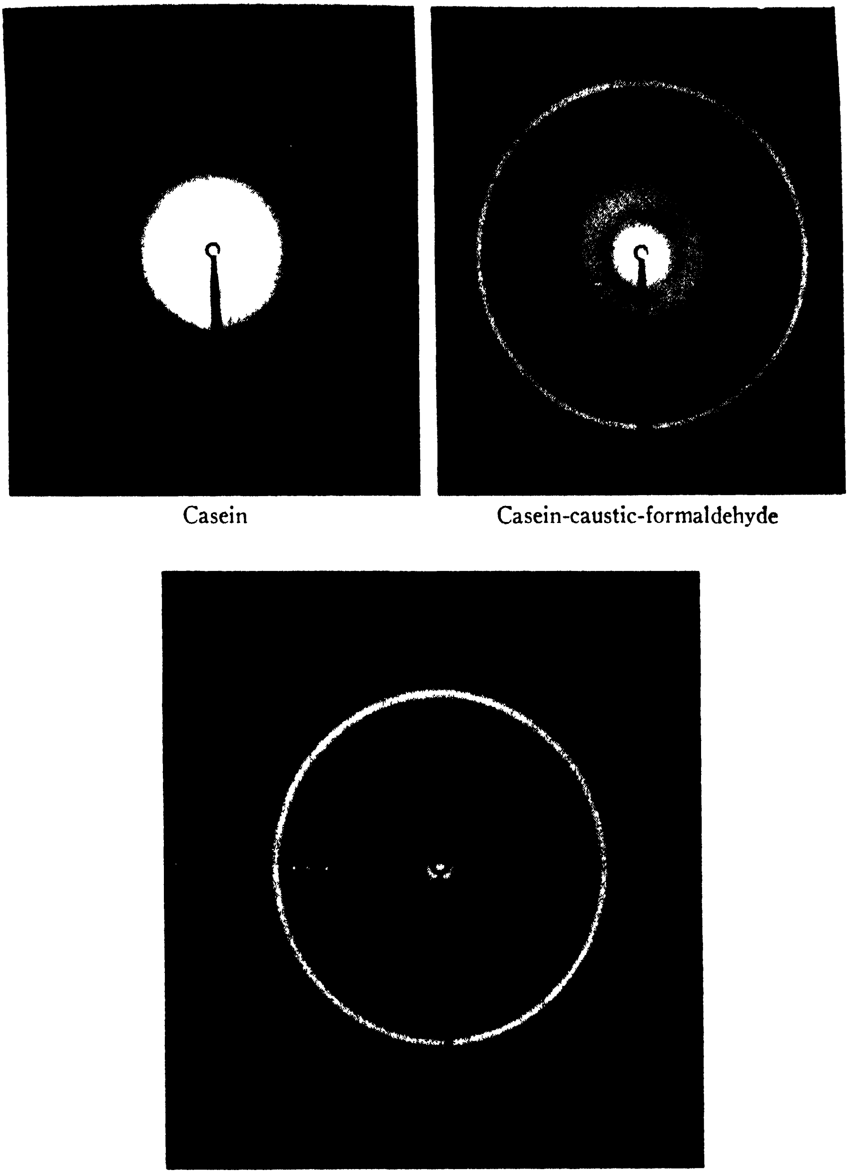
Until recently it has generally been believed that water is essential for the plasticization of protein plastic material. It has also long been recognized that the greatest weakness of protein plastics is their hygroscopicity; many attempts have been made to check or correct it while

plasticizing with water. It has now been shown[40, 130a] *that protein material possesses flow properties even when the water normally present has been reduced to the point where it could not possibly be considered* a plasticizer. Obviously this opens up a new field for the development of a non-hygroscopic protein plastic. There must exist polar molecules, immiscible with water, that can be worked into the protein molecule so that they will be held as the water has been, and will plasticize the protein. To find such materials and to work out their application to protein products is very worth while, and difficult enough to be interesting.

It may be of interest to note the difference in the soft plastic formed from acid and from rennet caseins. Acid caseins give an excessively soft, flabby plastic that is difficult to handle through the process of manufacture, and yield a product that is inclined to be comparatively brittle. Acid caseins are comparatively low in ash (1.5 per cent), containing practically no lime, whereas rennet casein is high in ash (7.5 per cent), with lime constituting about half the ash. It has therefore been held that the structure of the two caseins is different, causing the difference in the reaction with water. There has been little information to support any theory to date, except the fact that a spoilt rennet casein tends to produce a plastic similar to the acid casein; but some work has been started along this line,[247] and there may be data available before long on which to base a more definite and satisfactory theory. Examination of these two plastics comparatively with the infrared spectrograph might also yield some interesting and valuable results.

The reaction on which the practicability of protein plastic materials rests, *i.e.*, the protein hardening, or more specifically in this case, casein-formaldehyde, has not been understood.[175, 349] In lieu of something more definite, a condensation reaction, as suggested by Blum[53] has enjoyed wide acceptance. Much work is being done to obtain data on which more satisfactory deductions may be based, not only with regard to the hardening of galalith,[105] but also with regard to serum,[176] blood,[170] etc.

The Sørensen titration[153, p. 327] for the carboxyl groups in amino acids depends upon a condensation reaction with formaldehyde to block off the amino groups. Clark and Shenk,[82] studying the formaldehyde reaction on a number of proteins by means of x-ray diffraction methods, found two strong rings produced at 3.9 Ångstroms and 2.6 Ångstroms in the case of every protein examined, with the exception of silk fibroin. Casein was not included in this study but was later examined by Clark and his associates, with some interesting results. As shown in Figure 28, characteristic ring spacings appear in casein treated with formaldehyde under certain circumstances, although these ring spacings do not appear in the original casein. These spacings were at first attributed to the



Casein

Casein-caustic-formaldehyde

Paraformaldehyde

Courtesy of George L. Clark and associates

Figure 28.—X-ray photographs of casein, the product obtained by treating casein with sodium hydroxide and formaldehyde, and paraformaldehyde.

casein-formaldehyde complex. Examination of commercial casein plastic material, however, revealed an x-ray pattern more nearly like that of casein, in which the ring spacings attributed to the casein-formaldehyde complex were absent. To produce the characteristic ring spacings it is necessary to treat the casein with caustic either before or during the treatment with formaldehyde. It was later found that paraformaldehyde, which may be formed by polymerization of formaldehyde in alkaline solution, gives practically the diffraction spacings at first attributed to the casein-formaldehyde complex.

Tuckey[375] applied the x-ray diffraction method to the examination of various reactions with cheese, including that with formaldehyde. He found that when ripened cheese is treated with formaldehyde the ring at 3.83 Ångstroms becomes much more intense, while that at 2.6 Ångstroms is practically unaffected. He concluded that, since ripening of cheese is essentially hydrolysis, it is possible that "the diffraction interferences of the cheese samples are a result of amino acids liberated." To check this conclusion, he investigated the x-ray diffraction patterns of 19 amino acids, only three of which, isoleucine, aspartic acid, and proline, gave diffraction patterns at approximately 3.85 and 2.63 Ångstroms. He concluded that there is indicated either a reaction between some or all of these three amino acids and formaldehyde, or the liberation of one or more of them in the free state.

Astbury and Lomax[22] found that denaturization of protein apparently tends to break away side chain interferences, sharpening the backbone reflection and forming parallel bundles. Denatured proteins are known to be more water resistant. This opens up a very interesting field for research. It has been shown that proteins are probably made up of long filaments of polypeptide chains, held together by primary valence bonds, cross linkages, etc., and carrying side chains in which rests the reactive part of the molecule. By interreaction with one another, the side chains frequently produce a tangle in which the character of the backbone or polypeptide filament is largely lost. If, by proper and controlled denaturization these interfering side chains could be split apart and the bundle of filaments liberated and oriented, it should be possible to produce with this material not only textile fibers[21] but also plastics of superior strength.

A beginning has possibly been made in this direction. Recently it has been shown that it is possible to prepare a fully hardened and thermoplastic body from casein or other protein material,[61] especially soybean protein, by treatment with an aldehyde or other hardening agent. With soybean commercial protein, formaldehyde is taken up to about one per cent, forming a perfectly thermoplastic body with moisture reduced to 5 per cent or less. The protein-formaldehyde complex molds readily under heat and pressure with about 10 per cent moisture con-

tent, and the object comes finished from the die. The material is more water-resistant than the average casein plastic material and possibly a little more brittle. The brittleness can be corrected comparatively easily by incorporating the proper amount of plasticizing agents. Polyfunctional alcohols, especially ethylene glycol and furfuryl alcohol, have been found to give promising results. Water-resistance has been sharply increased, for example, by incorporating but one per cent of aluminum stearate in the powder before molding. The hardening reactions are being studied from every angle possible and it is hoped that from this study may be gained some fundamental information which may even bear on the structure of the protein molecule.

This work has already uncovered some interesting differences in hardening agents and it is planned to follow it further. In this connection, crotonaldehyde, propionaldehyde, chromium salts, and furfural are of especial interest; only the last of these has received much attention.[140, 185, 186]

The leather industry is built around a tanning or hardening reaction of the protein, collagen, that appears in many ways analogous to the hardening reaction for protein plastic material. Although most leather is chrome- or bark-tanned, formaldehyde and alum are used principally for white leather. Formaldehyde tan is a true tan and fairly permanent to washing or soaking in water. Alum tan, on the other hand, is a temporary tan and is readily washed or soaked out with water, leaving raw hide. As has already been pointed out, waste alum-casein can be returned to the process and reworked. This is not the case with formaldehyde-casein as produced in the casein plastics process. More general study of the ample literature on the tanning reactions by those interested in the development of protein plastic material is to be recommended, as there are doubtless numerous helpful analogies to be discovered.

There is a possibility of wider application in the protein plastics industry of the alum hardening reaction. As has been shown, acid caseins do not make satisfactory casein plastic material because they plasticize excessively in process and finish rather too brittle. It is quite possible that the proper percentage of a soluble aluminum salt mixed with the acid casein powder might lessen the first objection and correct the latter. If this proved to be the case, a considerable economy could be effected because acid caseins are much less expensive and are more widely available than rennet casein.

Properties and Characteristics of Casein Plastics

From its general reactions and probable chemical composition, casein plastic material may be considered an artificial horn.[162] It is difficult to ignite and chars rather than burns, emitting the characteristic odor

of burnt feathers, the recognized qualitative test for organic nitrogen. It is softened somewhat by moderate heat (190 to 212°F.), but not to the extent of nitrocellulose plastics, and it becomes rigid again much more rapidly on cooling. It is therefore possible to bend and emboss casein plastic material to some extent, but not to mold it. It is possible to emboss buttons from blanks of casein plastic material by placing the blanks in a hot mold in a press, closing the press very slowly to allow ample time for the blanks to become heated through before pressure is applied, and pressing and chilling under pressure, but it has not proved commercially important. The process is too slow, and the percentage of rejects high because of ragged edges; furthermore, buttons produced in this way tend to lose their shape and revert to flat discs if subjected to heat or moisture.

The translucent and transparent material has a specific gravity of approximately 1.35,[333] the opaque material being higher in proportion to the quantity and specific gravity of the filler used. The natural color is cream, which is a good base for other colors. Consequently the material is furnished in a great range and variety of colors and color effects, from light coral and turquoise shades to sapphire, amethyst, and jet black; in solid colors and in various horn effects; in mottled or streaked colors, tortoise shell, jade, cloudy amber, horns, etc. The dyes used are usually acid or special[198] colors and are reasonably light-fast. The almost unlimited possibilities in color production and the non-inflammability of the material are the two strongest and most generally used sales arguments of casein plastics manufacturers. In addition, the material is fairly tough and strong, having an average modulus of rupture of approximately 15,000 pounds per square inch.

Possibly because casein plastic material is fairly easily machined, drilled, etc., the mistake has frequently been made[147] of attempting large-scale production with it on machines and by methods designed for other materials. The fact is that the material is very hard on the cutting edges of tools, possibly because it is a very poor conductor of heat. It is therefore not very practical for working on high-speed automatic or semi-automatic machines. Working directions for bending, gluing, polishing, etc.[7, 385] may be found in the literature and both the Internationale Galalith Gesellschaft and Erinoid publish pamphlets of general directions. Occasionally special directions for a specific operation[195] may be found.

The material readily takes a lustrous and pleasing finish. In the older method the surface was prepared by "ashing" with powdered pumice stone and water, in tumbling drums or barrels for small and regularly shaped objects, and on rag buffing wheels for larger or irregularly shaped pieces. With the surface reduced to smooth uniformity, it is comparatively easy to produce an excellent polish by means of some

form of tripoli, wax, or rouge in tumbling barrels containing a number of wooden pegs with the wax, or by means of the rag buff. This polish is fairly permanent provided that the material is not scarred and scratched by rubbing against itself or against materials harder than itself, and provided that it is not subjected to frequent surface dampening, as for example handling with damp hands. In the latter event, the surface in time will be found covered with a network of fine surface fractures which do not affect the strength of the material appreciably but do destroy the beauty of the polish.*

The more recent "dip" or "chemical" polish[242] consists essentially[79, 380] of a bath of alkaline sodium[80] or calcium[137] hypochlorite. The articles to be polished are placed in such a bath heated to just below 212°F. and kept there from 15 to 20 minutes with occasional stirring. When they are removed and washed the surface is found to be soft, but on drying it sets to a glaze. This at the same time gives a polish to the surface and, it is claimed, increases the water-resistance of the material. If dye is applied while the surface is soft, deeper penetration results.

This development is of considerable importance to the button industry, particularly that section supplying casein plastics buttons to the clothing industry in competition with vegetable ivory and Bakelite. Not only is a material saving in cost effected over the method of wax polishing in tumbling barrels, but the finish produced by the dip polish stands up under the conditions of the steam clothes press used in most cleaning and pressing establishments in the United States, and keeps its luster and polish unimpaired. This is not the case with the wax-polished casein plastic button.

A number of interesting methods of producing colored or ornamental surface effects have been worked out for application, usually after the material has been wax-polished. For coloring, it may be soaked in an aqueous solution of a basic dye[17] and fixed with alum; it may be treated with an aqueous suspension of a dye[196] insoluble in water; it may be treated with electrolytes with colored ions or with ions that produce colored products on reacting with protein.[204] Or it may be dyed by soaking in an aqueous acidified solution of an acid dye. These colors are all confined to the very thin surface layer so that two-tone effects may be produced by cutting designs on the dyed material. It may also be etched[41] by application of an aqueous acetone solution of an alkaline

* The junior editor (F.L.B.) has two desk pens with handles made of casein plastic material that were given to him twelve years ago by Dr. Brother. One of them, which has been in almost daily use, exhibits the loss of polish and the fine surface fissures (checking) described, though a good magnifying glass is required to observe them and they remained unobserved until the paragraph above was read. The other, which has been used only infrequently, is still brightly polished and apparently free from checking.

hydroxide or carbonate. It is claimed that a metallic coating will be deposited if the article is treated with an aromatic reducing agent, such as *p*-aminophenol[130] and then immersed in a water solution of a reducible metallic salt, such as silver.

The most serious weakness of casein plastic material is a direct legacy from casein. The readiness with which casein absorbs water is of considerable importance in the manufacture of casein plastic material as it has been made up to the present time; but this tendency, though checked to a great extent by the hardening reaction, eliminates it from a number of fields and seriously restricts its application in others. Wernicke[385] gives the following figures on water absorption of galalith, as indicated by increase in weight when submerged in water: after 24 hours, 22.5 per cent; after 5 days, 31 to 32 per cent; after 6 weeks, 31 to 32 per cent. These may be considered a little high on the average.

The material not only absorbs moisture if soaked in water but it also "breathes" with changes in relative humidity of the air. Absorption of large amounts of water when soaked causes softening and swelling; drying causes hardening and contraction, a volume change that tends to weaken the material and cause it to fracture. Sudden temperature and humidity changes in many parts of the United States, as well as extreme variation from one place to another, render casein plastic material unfit for use in combs, which is a large European outlet.

Sensitivity to humidity changes also renders it inapplicable in large panels or long rods because they are certain to warp badly. The volume change also makes it inapplicable where accurate dimensions are required, for example, for sliding or friction fits. On days of high humidity it swells sufficiently to tighten the sliding part too much, and on dry days it shrinks enough to permit the sliding part to fall off. This defect, together with the tendency to loss of polish by surface fractures caused by handling, has practically eliminated the material from the pen and pencil field. Obviously this same weakness has restricted its use in the electrical field to interior insulation,[68] and comparatively little of that. If kept dry, casein plastic is an excellent electrical insulator but a very small absorption of moisture causes its dielectric constant to drop sharply.

The following list of properties of casein plastic material, taken from a compilation of the properties of various plastics given in *Modern Plastics* for October 1938, is given for convenient reference although the author can give no assurance that the data are reliable. The estimate of water absorption, for example, seems much too low.

Molding qualities	poor
Molding temperatures, degrees F	200 to 225
Molding pressures, pounds per square inch	2000 to 2500
Specific gravity	1.35
Specific volume, cubic inches per pound	20.5

Tensile strength, pounds per square inch	7600
Impact strength, foot pounds per inch	1.0 (Izod)
Modulus of elasticity, pounds per square inch times 10^5	5.1 to 5.7
Thermal expansion, 10^{-5} per degree C.	8
Water absorption, per cent by weight in 48 hours	3 to 7
Breakdown voltage, 60 cycles, volts per mil (instantaneous)	400 to 700
Dielectric constant, 10^6 cycles	6.15 to 6.8
Power factor, 10^6 cycles	0.052
Hardness, 2.5 mm. ball, 25 kg. load, depth in 1/200 mm.	23
Softening point, degrees F.	200
Burning rate	very low
Effect of age	hardens slightly
Effect of sunlight	colors may fade
Effect of weak acids	resistant
Effect of weak alkalies	softens
Effect of strong acids	decomposes
Effect of strong alkalies	softens
Effect of organic solvents	resistant
Machining qualities	good
Clarity	opaque or translucent
Color possibilities	unlimited

Applications and Possibilities

It has been shown that casein plastic material in sheets and rods does not enter into the American industrial picture to any extent and there is good probability that it will be even less important in the future. Some statistics may be of interest to give at least some idea of the magnitude of this business.

The world production of casein[143] was 60,000 tons in 1932; 70,000 tons in 1936. Two-thirds of the casein produced in the United States, by one of the three largest producers, went into the paper industry. The world production of casein plastic material in 1930 was of the order of 10,000 tons,[259, 264, 333] while in the United States in 1931 there was produced but 1250 tons and only 6 tons[366] were imported. The import figures for 1934 increased only to 6.63 tons.[41] Although no figures are available, there is no doubt that the domestic production increased sharply between 1931 and 1934, but, as has been shown, this was because of activity in the button industry, not in the casein plastics industry as such. To the 6 tons of imported sheets and rods can be added a maximum of 50 tons, and some of this doubtless goes into the manufacture of very fancy dress buttons and buckles. Hence the annual consumption of casein plastics in the United States as sheets and rods is only 56 tons.

It is apparent that the only important development in the industry has been more intensive application to the manufacture of buttons. This application is not new nor exclusively American, an estimate for 1926 showing that approximately 55 per cent of the world's supply of buttons was made of casein plastic material.[10] The American development brought the material and methods of manufacture into tune with

American industrial methods, so that it is possible to fabricate the casein plastic buttons cheaply and rapidly enough to compete successfully with vegetable ivory and bone in the lower price field. How successful this has been is shown on the production chart of one of the largest of these companies. There was practically no production in 1931 and 1170 tons in 1935, which was nearly the total amount of casein plastic material consumed by the entire country in 1931. As has been shown, there are seven companies fabricating buttons or button blanks of casein plastic material. The total production at the present time must be between 4000 and 5000 tons annually.

Until the hygroscopicity of the material is greatly reduced it is really suitable, as Dunham[120] states, for buttons and buckles only. The discovery that the hardened material with reduced water content is thermoplastic makes it possible to correct this fault and indicates many fields of possible industrial application.

The trends for some years back have been in this direction, as evidenced by the experiments to discover plasticizers and hardening agents, and to produce molding powders, with and without other synthetic resinous materials. With the knowledge that the hardened, dehydrated material is thermoplastic emphasis can now be directed to finding plasticizers by means of which any degree of elasticity or pliability, together with increased water-resistance, may be imparted to the finished material. It has been shown that a small percentage of an agent, such as aluminum stearate, has a marked effect in this direction.

Recent work has demonstrated that thermoplastic formaldehyde-hardened soybean protein, containing one per cent formaldehyde and not over 3 per cent moisture, may be mixed with phenolic or urea-formaldehyde molding compounds to produce molding powder with thermosetting properties. A mixture of equal parts of the hardened protein and regular phenolic-wood flour molding compound may be molded at 330°F., cured 2 minutes, and removed from the die without any chilling. The absorption of water by these mixtures is much less than that of the hardened protein alone and not much more than that of the resin. A mixture of equal parts of thermoplastic soybean protein hardened with formaldehyde and commercial urea-formaldehyde molding powder produced a molded product with only 1.7 per cent absorption of water in 24 hours' immersion.[61]

There has recently been developed a new and more rapid method of molding plastic material, known as the injection process.[354] There are a number of modifications possible, for example, provision for heat setting,[71] provision for condition of the plastic for introduction,[94] etc., but in general it consists in introducing the thermoplastic material into a machine[284] in which it is heated and then forced hot into compara-

tively cold dies to conform and set. To function, this requires a perfectly thermoplastic material that flows very freely. Properly plasticized, hardened protein plastic material should fulfill this requirement and produce as good finished articles in every respect as cellulose plastics at about half the cost, if a water-repellent plasticizer can be found that will make the casein sufficiently fluid. The future of such a material is not predictable, but the probability is that it would shortly take a major position among the industrial plastic materials.

Appendix to Chapter 7

Synthetic Textile Fibers from Casein*

Production

The development of synthetic fiber from casein is of such recent date that anything more than a brief discussion of its present status and potential future is impossible. Although it has been commercially produced in Italy for three years, casein wool is still in a state of flux; changes and improvements have been made so rapidly during the last few months that any data obtainable are likely to be unreliable, and to be soon out of date. For example, it is reported[368a] that, whereas the strength of the Lanital fiber was formerly (1935)[44] only 10% that of a wool fiber of comparable size, it has been improved to such an extent that it is now (1938) 85% as strong as wool. However, in view of the wide interest in this material, particularly in Europe, and of its promising future in the textile world, it was thought advisable to present the information given in this section.

It seems that the first practical attempts to spin casein filaments were carried out in Germany by Dr. Todtenhaupt,[221, 371, 371a] although Eggert[122a] has published a patent review giving priority to Millar in 1895.[257a] The fibers were produced by dissolving dry casein in a suitable solvent, probably dilute sodium hydroxide; but they did not resemble wool, being hard, brittle and of poor durability.[55a] Transformation of this unpromising product into a pliable synthetic fiber having many of the properties of wool was the work of Dr. Antonio Ferretti.[12, 18, 289] He investigated this problem from 1924 to 1935, when the Italian rayon company Snia Viscosa bought his patent and undertook to produce artificial wool on a large-scale basis. The fiber was named "Lanital," meaning "Italian wool." His knowledge of the tech-

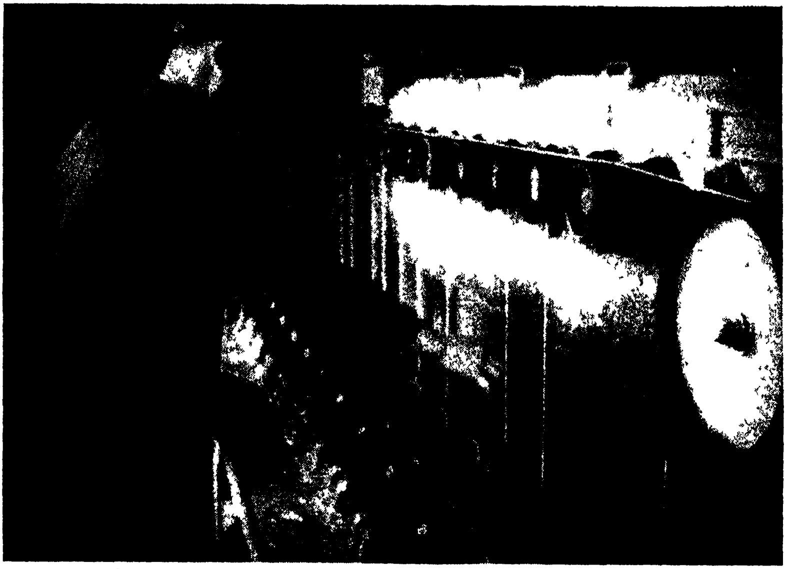
* Compiled from reliable sources by G. G. Hawley, Editorial Department, Reinhold Publishing Corporation.

nic of rayon manufacture greatly aided Dr. Ferretti; and it will be seen how similar the two processes are.

Licenses have since been granted in Canada, France, Belgium, Holland, Poland, Germany, Czechoslovakia and Japan[14]; and Snia Viscosa is now building a new plant for Lanital production.[368a] In 1936 Italian output was 300 tons; in 1937 1,200 tons[325] had been produced up to September. In December, 1937, the United States began importing Lanital through the American agents of Snia Viscosa; but it has not as yet found wide application in this country. Italy's capacity to produce Lanital is limited by her supply of casein; but for this she is not at present dependent on domestic sources, purchase from Holland and Denmark being little if any more costly.

Processing

The Italian process appears to utilize a dispersion of casein in aqueous alkaline solution to which is added some such agent as carbon di-



Courtesy of 1937 Universal Newsreel from C & W, Inc. and of Meyer & Marks Yarn Co., New York

Figure 28a—Filaments of Lanital as they are forced through spinnerets underneath solution bath. Pumps in foreground regulate speed of the stream, thereby determining fineness.

sulfide.[24, 331] As in rayon production the solution is then extruded, coagulated in sulfuric acid (see Fig. 28a) and after drying, treated with formaldehyde. Suitable plasticizers have been found[304] which

render the fiber elastic and resistant to water and fire. It is probable[55a] that formaldehyde condenses with the amino groups of the casein, thus:

$$R—NH_2 + CH_2O \longrightarrow R—N—CH_2 + H_2O$$

The Italian producers state that one unit weight of casein yields one unit weight of Lanital. It can be cut to any staple, 4½" being the average, as compared with 6" to 7" for wool; and the fibers can be spun to the finest counts. Several descriptions of the process have been published.[142, 268]

Whittier and Gould of the U. S. Department of Agriculture, Bureau of Dairy Industry, have recently applied for patents for a slightly different method of producing casein wool.[13a, 153a] "To make the fiber, casein is softened in water and dissolved in a solution of caustic alkali. It becomes a thick, sticky mass and is carefully worked into the proper consistency by aging, the addition of modifying agents, and by dilution. The mass is then forced through multiple spinnerets of the kind used in making rayon. The fibers are separated and hardened in an acid bath containing formaldehyde and modifiers."

Important proposed modifications of this process are the addition of a 10 per cent dispersion of casein to viscose;[131] the development of synthetic fibers from collagen[135, 238, 346] and from animal tendons.[326] It has also been proposed to use an aqueous dispersion of vegetable proteins[75] and of casein[76] in urea solutions for production of fibers.

Composition and Properties

The chemical composition of synthetic wool very closely approximates that of natural wool:[56, 99, 142, 268, 285, 318]

Element	*Lanital*	*Natural wool*
Carbon	53 %	49.31%
Hydrogen	7.5	7.57
Oxygen	23	23.66
Nitrogen	15	15.86
Sulfur	0.7	3.60
Phosphorus	0.8	0.0

It will be noted that the only significant differences are that Lanital has a comparatively low sulfur content and that it contains a small percentage of phosphorus.

There seems to be some difference of opinion regarding the smoothness of casein fiber, some authorities[325] stating that microscopic examination shows it to be very smooth, and others[55a] that it is rather rough, or at least "not completely smooth." It is agreed, however, that in a microscope Lanital fiber is difficult to distinguish from rayon.

As to the tensile properties of Lanital little can be reported authori-

tatively. As previously mentioned, its dry strength is now said to be 85% that of natural wool. In company with rayon, in its earlier stages, at least, Lanital has the unfortunate property of losing a great part of its strength when wet, the percentage of loss varying between 50[55a] and 60.[325] However, it regains its original strength on drying.[325] It has an elongation of 84%[325] but is only slightly elastic, the regain amounting to 15.2%, at 65% relative humidity.[56a] At this humidity its moisture content averages 11.7%,[55a] which is higher than that of wool.[372, 388]

Dyeing

Like wool, Lanital is an amphoteric material, but it dyes more easily and more evenly than wool.[56a] The best success has been obtained with acid dyes, which give a deeper shade than in the case of wool. Dyeing should be performed at about 70°C., at which temperature Lanital is unaffected.[55a] If the temperature used is very greatly in excess of 70°C. the dyes will tend to bleed; this is especially true of alkaline dyes. Moreover, exposure to temperatures near or above the boiling point of water tenderizes Lanital fabric. Strength lost in this way can never be regained. Only dyes known to be extremely fast should be used.

Mixtures of Lanital and Wool

Lanital fabric as such can be used for some purposes; but its future lies in the extent to which it can be utilized in mixtures with wool. Usually blends are composed of 50% wool and 50% Lanital; less frequently 75% Lanital and 25% wool; these give satisfactory products for felting.[301, 325] Such blends are suitable for uniforms, underclothing and miscellaneous men's wear; they have high resistance to creasing, but reflect the weakness of Lanital when wet. Moreover, Lanital is not susceptible to attack by moths, and is as warm as wool.[325] However, it appears to be as susceptible to attack by bacteria as are other forms of casein. Two Dutch chemists, Smit and van der Heide, state, "It was found that nearly all casein-splitting microörganisms isolated from soil or manure or gathered by infection from ordinary atmosphere are able to attack (synthetic) wool." [387a]

Distinguishing Tests

Lanital may be distinguished from wool qualitatively by a combustion test; it burns somewhat more readily than wool, does not swell, and gives off an odor very similar to that of burning wool. Methods of distinguishing between Lanital and wool with the aid of a microscope[234, 240, 299] and by ultraviolet fluorescence[388] have been described. However, a chemical test is likely to be more accurate. If Lanital fibers

are treated with a 10% solution of NaOH at room temperature for a few minutes, then washed and immersed in a 1% solution of methylene blue, and rinsed, the Lanital turns intensely blue, whereas the wool is only slightly tinted to a greenish-blue.[56a]

A quantitative method developed by DaSchio[100] has also been described[162a] based on the difference in solubility in dilute sodium hydroxide of Lanital and natural wool. Cut up 2 grams of Lanital-wool mixed fabric and stir into 100 cc. of 20% NaOH; maintain at 30°C. for 3 hours and then filter on a 100-mesh sieve. This process dissolves all the wool, the Lanital remaining as residue. Wash the residue with water, then with dilute acetic acid; then dry to constant weight at 105°C. Since 9.5% of the Lanital will have dissolved[56a] in the NaOH, the residual weight of Lanital must be multiplied by 1.09 to obtain the weight of this fiber in the original 2 grams of mixture. After the percentage of moisture in the original fabric is found by drying at 105°C., the proportion of wool can be calculated by difference.

The solubility of casein fiber in NaOH is reported[56a, 162a] as follows:

Immersion in 20% NaOH at 30°C. (hrs.)	Loss of weight of Lanital (%)
1	7.7
2	10.4
3	13.3
4	15.4
5	18.5

The effect of temperature on degree of solubility in caustic is given as

Temperature of 20% NaOH (°C.)	Loss of weight of Lanital in 3 hrs. (%)
20	8.2
30	9.5[56a]
40	22.[illegible] (?)

It must be emphasized, however, that these figures cannot be accepted without considerable reservation. For example, until very recently the loss of weight after 3 hours' immersion at 30°C. was thought to be 12.3%.[162a] The latest value of 9.5% suggests that all other solubility values quoted here are probably too high.

It is still too early to predict with confidence the future of this most recent casein product. Great interest is being displayed in its manufacture in various European countries besides Italy; and it is probable that it will soon be under way in the United States. The possibilities of combining it with viscose rayon production, the likelihood of early technical improvements which will eliminate or minimize its weaknesses, and the present wide interest in synthetic products of all kinds[156] seem

to indicate that artificial wool fiber will eventually attain the commercial importance of rayon.

References

1. Akalit Kunsthornwerke A.-G., Austrian Pat. 132,413 (Mar. 25, 1933)
2. Albert, Kurt, G.m.b.H., Chim. Farb., French Pat. 784,158 (July 22, 1935)
3. Albert and Berend, French Pat. 436,720 (Apr. 3, 1912)
4. Alden, Canadian Pat. 154,062 (1914)
5. Allgemeine El. Ges., British Pat. 445,839 (not granted)
6. Anonymous, *Kunststoffe,* **2,** 225 (1912)
7. Anonymous, *Kunststoffe,* **9,** 208 (1919)
8. Anonymous, *Rev. gen. mat. plastiques,* **2,** 41 (1926)
9. Anonymous, *Kunststoffe,* **17,** 67 (1927)
10. Anonymous, *Le caout. et la gutta-percha,* **24,** 13836 (1927)
11. Anonymous, *Ind. Chemist,* **9,** 193 (1933)
12. Anonymous, *U. S. Dept. of Commerce, E. and T. Notes,* 119 Rome (1935); Ferretti, A., British Pat. 483,731 (Apr. 21, 1938); 483,807; 483,808; 483,809; 483,810; French Pat. 813,427 (June 1, 1937)
13. Anonymous, *U. S. Dept. of Agriculture, Daily Digest,* **59,** No. 42 (Nov. 18, 1935); **60,** No. 53 (Mar. 4, 1936)

13a. Anonymous, *U. S. Dept. of Agric., Daily Digest,* **70,** No. 32 (Aug. 16, 1938); *Ind. Eng. Chem., News Ed.,* **16,** 525 (1938)

14. Anonymous, *Oil, Paint & Drug Reptr.,* July 13, 1936; *Rayon Melliand Textile Monthly,* **17,** No. 9, 44 (1936); *Internatl. Review on Timber Utilization,* **15,** 15 (Feb. 1938); *Ind. Eng. Chem. News Ed.,* **16,** 195, 232, 363, 365, 402, (1938)
15. Apple, L. A., U. S. Pat. 1,765,819 (June 24, 1930)
16. Artem'ev, N. I., U.S.S.R. Pat. 31,611 (Oct. 31, 1933)
17. Artus, F. v., *Gelatine Leim Klebstoffe,* **3,** 136 (1935); **6,** 75 (1938)
18. Astbury, W. T., *J. Soc. Chem. Ind.,* **49,** 441 (1930)
19. Astbury, W. T., "Fundamentals of Fibre Structure," London, Oxford Univ. Press, 1933.
20. Astbury, W. T., *J. Soc. Chem. Ind.,* **53,** 979 (1934)
21. Astbury, W. T., Dickinson, S., and Bailey, K., *Biochem. J.,* **29,** 2351 (1935)
22. Astbury, W. T. and Lomax, R., *J. Chem. Soc.,* **1935,** 846
23. Badarelli, I., *Rev. gen. mat. plastiques,* **9,** 152 (1933)
24. Bader, W., *Kunstseide,* **18,** 117 (1936)
25. Bähr, A., German Pat. 385,783 (Nov. 28, 1923)
26. Bakelite Corpn., French Pat. 670,000 (Feb. 21, 1929)
27. Bandman, A. O., Australian Pat. 100,119 (Jan. 28, 1937)
28. Barg, E. I., *Plasticheskie Massui,* No. 2, 29 (1935); *Chem. Abst.,* **31,** 176
29. Barnett, U. S. Pat. 865,005 (Sept. 3, 1907)
30. Bartels, A., British Pat. 21,750 (Sept. 19, 1910); French Pat. 420,543 (Jan. 23, 1911, 1st ed.)
31. Bartels, A., French Pat. 420,543 (Apr. 6, 1911, 2d ed.)
32. Bartels, A., U. S. Pat. 1,171,382 (Feb. 8, 1916)
33. Bartels, A., U. S. Pat. 1,211,526 (Jan. 9, 1917); Swiss Pat. 72,634 (June 16, 1916)
34. Bartels, A., Norway Pat. 27,814 (Apr. 10, 1917); U. S. Pat. 1,320,666 (Nov. 4, 1919)
35. Bartels, A., British Pat. 13,203 (Sept. 15, 1915); Swiss Pat. 72,635 (June 16, 1916); U. S. Pat. 1,209,539 (Dec. 19, 1917); 1,375,624 (Apr. 19, 1921)
36. Bartels, A. and Miech, U. S. Pat. 1,560,368 (Nov. 3, 1925)

37. Bartels, A. and Miech, U. S. Pat. 1,623,085 (Apr. 5, 1927)
38. Bassett, H. F., U. S. Pat. 1,360,356 (Nov. 30, 1920); Bauer, R. and Mauthe, G., see I. G. Farbenind. A.-G., ref. 194
39. Beau, M., *Lait,* **13,** 325 (1933)
40. Beckel, A. C., Brother, G. H. and McKinney, L. L., *Ind. Eng. Chem.,* **30,** 437 (1938)
41. Becker, F., German Pat. 524,244 (July 29, 1926)
42. Berend, L., U. S. Pat. 952,724 (Mar. 22, 1910)
43. Berend, L., U. S. Pat. 1,040,850 (Oct. 8, 1912)
44. Bergen, W. v., *Bull. Natl. Assoc. Wool Mfrs.,* **65,** 272 (1935); *Am. Dyestuff Reptr.,* **25,** 146 (1936); *Rayon and Melliand Textile Monthly,* **17,** 39, 75 (1936); *J. of the Textile Institute,* **27,** No. 4, A154 (Apr. 1936)
45. Berges, C. & N., Machinienfabrik., British Pat. 446,021 (Apr. 23, 1936)
46. Berlin, H., U. S. Pat. 1,988,475 (Jan. 22, 1935)
47. Besana, G., *Matière Plastiche,* **3,** 181 (1936)
48. Betts, P. W. F. G., U. S. Pat. 893,129 (July 14, 1908)
49. Billiez, R. T., French Pat. 790,106 (Nov. 14, 1935)
50. Birrell, T. L., *Chemistry & Industry,* **56,** 544 (1937); *Trans. Inst. Plastics Ind. (London),* **6,** 137 (1937); *Modern Plastics,* **16,** No. 2, 17 (1937)
51. Blücher and Krause, German Pat. 289,597 (Feb. 13, 1915); U. S. Pat. 1,238,-528 (Aug. 28, 1917); 1,319,666 (Oct. 21, 1919)
52. Blücher, H., "Plastiche Massen," S. Heizel, Leipzig (1924)
53. Blum, F., *Z. physiol. Chem.,* **22,** 127 (1896)
54. Boerma, German Pat. 241,887 (Jan. 16, 1910)
55. Bonte, F., *Kunststoffe,* **27,** 64 (1937)
55a. Bodenbender, H. G., *Silk and Rayon,* **12,** 456 (1938)
56. Borgetty, H. C., *Am. Dyestuff Reptr.,* **25,** 538 (1936)
56a. Borgetty, H. C., *Am. Dyestuff Reptr.,* **27,** 156 (1938)
57. Bormans, E., U. S. Pat. 1,885,563 (Nov. 1, 1933)
58. Brigando, J., *Lait,* **13,** 657, 843, 965, 1098, 1230 (1933)
59. British Celanese Ltd., British Pat. 453,833 (Feb. 25, 1936)
60. British Thomson-Houston Co., British Pat. 444,626 (Mar. 20, 1936)
61. Brother, G. H. and McKinney, L. L., *Modern Plastics,* **16** No. 1, 41 (1938); *Ind. Eng. Chem.,* **30,** 1236 (1938); *British Plastics,* **10,** 248 (1938)
62. Brubaker and Thomas, U. S. Pat. 2,009,432 (July 30, 1935)
63. Busse, H., German Pat. 523,947 (Feb. 29, 1928)
64. Buswell, A. M., Deitz, V., and Rodebush, W. H., *J. Chem. Phys.,* **5,** 501 (1937)
65. Buswell, A. M., Krebs, K., and Rodebush, W. H., *J. Am. Chem. Soc.,* **59,** 2603 (1937)
66. Butterfield, U. S. Pat. 1,516,841; 1,516,842; 1,516,843 (Nov. 25, 1924)
67. Carpender, E. P., U. S. Pat. 1,740,573 (Dec. 24, 1929)
68. Carpenter, D. C. and Kucera, J. J., *Tech. Bull.* No. 230, *New York State Agric. Expt. Sta.* (*Geneva*), 16 (1935)
69. Carpzow *et al.*, German Pat. 591,540 (Jan. 23, 1934)
70. Castles, J. T., Jr., U. S. Pat. 2,036,195 (Apr. 7, 1936)
71. Celluloid Corpn., British Pat. 460,078 (Jan. 20, 1937)
72. Chandeysson, U., French Pat. 814,968 (July 3, 1937)
73. Charch, W. H., U. S. Pat. 2,065,792 (Dec. 29, 1936)
74. Chase, H., *Brit. Plastics,* 516 (Apr. 1936)
75. Chibnall, A. C., Bailey, K., and Astbury, W. T., British Pat. 467,704 (June 22, 1937); French Pat. 812,474 (May 11, 1937)
76. Chibnall, A. C., Bailey, K., and Astbury, W. T., British Pat. 467,812 (June 22, 1937)

77. Childs, E. E., German Pat. (1885); U. S. Pat. 307,179; 307,269 (Oct. 28, 1884)
78. Chollet, A. and Camus, A., *Lait*, **13**, 362 (1933)
79. Christensen, P. C., Canadian Pat. 355,416 (Jan. 21, 1936)
80. Christensen, P. C., U. S. Pat. 2,097,144 (Oct. 26, 1937)
81. Christmas, W. W., U. S. Pat. 1,612,098 (Dec. 28, 1926); 1,758,500 (May 13, 1930)
82. Clark, G. L. and Shenk, J. H., *Radiology,* **28,** 357 (1937)
83. Clemens, E., U. S. Pat. 1,819,811 (Aug. 18, 1931)
84. Clemens, E., U. S. Pat. 1,843,944 (Feb. 9, 1932); Reissue 19,379 (Nov. 27, 1934)
85. Clement and Rivière, "Matières Plastiques Soies Artificielles," Baillière et Fils, Paris (1924)
86. Clickner, F. H., U. S. Pat. 2,044,282 (June 16, 1936); Clickner, F. H., see Kraft-Phoenix Cheese Co., ref. 223
87. Coblentz, W. W., *J. Franklin Inst.,* **172,** 309 (1911)
88. Coleman, C. E., U. S. Pat. 2,082,618 (June 1, 1937)
89. Collarden, L., German Pat. 174,877 (Feb. 17, 1904); French Pat. 359,073 (Mar. 15, 1906)
90. Collet, R., French Pat. 734,335 (July 1, 1931)
91. Concannon, C. C., *U. S. Dept. of Commerce, Suppl. to Stat. Statements* No. 2814 and 2863 *and to "World Trade Notes on Chem. and Allied Prod."*
92. Concannon, C. C., *U. S. Dept. of Commerce, World Trade Notes on Chem. and Allied Prod.,* 12 No. 2, 28 (1938)
93. Cooper, C. A. and Hand, P. G. T., *J. Soc. Chem. Ind.,* **55,** 341T (1936)
94. Corin, P. G., Canadian Pat. 365,366 (Apr. 13, 1937)
95. Crane, P. W. and Fields, R. T., U. S. Pat. 1,956,564 (May 1, 1934)
96. Crane, P. W. and Fields, R. T., U. S. Pat. 2,083,557 (June 15, 1937)
97. Damgaard, P. P., British Pat. 22,644 (Oct. 7, 1913)
98. Dangelmajer, C., U. S. Pat. 2,101,574 (Dec. 7, 1937)
99. Da Schio, E., *Boll. assoc. ital. chim. tessile color.* **12,** 180 (1936)
100. Da Schio, E., *Rev. gen. mat. color,* **41,** 170 (1937)
101. Davidson, J. G. and Reid, E. W., U. S. Pat. 1,772,131 (Aug. 5, 1930); Canadian Pat. 302,083 (July 15, 1930)
102. Dejarme, N., *Rev. gen. mat. plastiques,* **13,** 63 (1937)
103. Delorme, J., *Rev. gen. mat. plastiques,* **11,** 375 (1935)
104. Delorme, J., *Rev. gen. mat. plastiques,* **12,** 14, 38 (1936)
105. Delorme, J., *Rev. gen. mat. plastiques,* **12,** 74 (1936); **14,** 109, 122 (1938)
106. Delorme, J. and Bluma, R., *Rev. gen. mat. plastiques,* **14,** 13 (1938)
107. Déribéré, M., *Rev. gen. mat. plastiques,* **10,** 57, 99 (1934)
108. Deutsche Hydrierwerke, A.-G., French Pat. 785,009 (July 31, 1935)
109. Deutsche Hydrierwerke, A.-G., French Pat. 792,989 (Jan. 14, 1936)
110. Deutsche Kunsthorn Ges., German Pat. 317,721 (Aug. 21, 1915)
111. Deutsche Kunsthorn Ges., German Pat. 368,569 (Mar. 30, 1916); Danish Pat. 22,433 (Sept. 10, 1917); German Pat. 366,958 (June 5, 1920); Swiss Pat. 76,685
112. Dodd, R., *The (London) Times,* Trade and Eng. Suppl. Mar. 28, 1931; Plastic and Allied Ind. Sect., p. 9
113. Dodd, R., *Trans. Inst. Plastics Ind.,* **5** No. 10, 58 (1936)
114. Dodd, R., *Chemistry and Industry,* **56,** 273 (1937)
115. Donley, H. C. and Rosinger, J., French Pat. 770,662 (Sept. 18, 1934)
116. Dubovskii, G. B., Russian Pat. 50,207 (Jan. 31, 1937)
117. Dumont, H., and Lück, W., German Pat. 619,532 (Oct. 4, 1935); Dumont, H., see Internatl. Galalith Ges., refs. 203, 204, 207

118. Duncan, F. S., British Pat. 429,523 (May 31, 1935)
119. Dunham, H. V., U. S. Pat. 748,709 (Jan. 5, 1904)
120. Dunham, H. V., *Plastic Products,* **9,** 101 (1933)
121. du Pont, E. I. de Nemours & Co., British Pat. 419,293 (Nov. 5, 1934)
122. Eberhard, U. S. Pat. 1,083,275 (Jan. 6, 1914); 1,095,979 (May 5, 1914)
122a. Eggert, J. W., *Gelatine Leim Klebstoffe,* **6,** 45 (1938)
123. Eisner, H., German Pat. 592,960 (Feb. 19, 1934)
124. Ellis, C., "Chemistry of Synthetic Resins," p. 415, New York, Reinhold Publishing Corp., 1935
125. Ellis, C., U. S. Pat. 2,075,276 (Mar. 30, 1937); 2,075,804 (Apr. 6, 1937)
126. Erasmus, L., Austria Pat. 784 (Oct. 18, 1909); German Pat. 257,814 (Oct. 26, 1909)
127. Eriksson-Quensel, L. and Svedberg, T., *J. Am. Chem. Soc.,* **58,** 1863 (1936)
128. Erinoid Ltd. and Leggat, A., British Pat. 354,837 (May 17, 1930)
129. Ernst, O., and Sponsel, K., U. S. Pat. 1,841,797 (Jan. 19, 1932); German Pat. 485,189 (Oct. 27, 1926); 530,934 (Sept. 13, 1927)
130. Eschingen, M. O., Austria Pat. 126,123 (Aug. 15, 1931); U. S. Pat. 1,890,645 (Dec. 13, 1932); 2,010,805 (Aug. 13, 1935)
130a. Esselen, G. J. and Scott, W. M., *Chem. Ind.,* **43,** 265 (1938)
131. Esselman, P., and Kösslinger, K., U. S. Pat. 2,059,632 (Nov. 3, 1936)
132. Fauquet, H. E., German Pat. 138,783 (July 25, 1901)
133. Fernbach, A., British Pat. 468,329 (July 2, 1937)
134. Frankenburger *et al.,* U. S. Pat. 1,756,943 (May 6, 1930)
135. Freudenberg, C., French Pat. 803,094 (Sept. 22, 1936); British Pat. 446,533 (May 1, 1936); 470,199 (Aug. 11, 1937)
136. Friedel, *Milchwirtschaft Ztg.,* **34,** 1680 (1929)
137. Frolov, S. I., U.S.S.R. Pat. 34,460 (Feb. 28, 1934)
138. Frood, H., British Pat. 176,405 (1920)
139. Fuhrmann, L. J., U. S. Pat. 2,006,736 (July 2, 1935)
140. Genin, G., *Rev. gen. mat. plastiques,* **7,** 89 (1931)
141. Genin, G., *Lait,* **16,** 1083 (1936)
142. Genin, G., *Lait,* **16,** 510 (1936); **17,** 949 (1937); **18,** 481 (1938)
143. Genin, G., *Lait,* **17,** 605 (1937)
144. Genin, G., *Lait,* **17,** 815 (1937); **18,** 45 (1938)
145. Gentile, M., and Weiss, S. W., British Pat. 350,993 (Dec. 16, 1929); U. S. Pat. 1,804,556 (May 12, 1931)
146. Glorith Kunsthornwerke Schiel & Co., German Pat. 527,262 (Aug. 7, 1926)
147. Glückner, *Kunststoffe,* **1,** 85 (1911)
148. Goldsmith, B. B., U. S. Pat. 965,137 (July, 19, 1910)
149. Goldsmith, B. B., U. S. Pat. 840,931 (Jan. 8, 1907); 964,964 (July 19, 1910); 1,027,122 (1912); British Pat. 14,098 (June 18, 1907)
150. Goldsmith, B. B., U. S. Pat. 1,076,417 (Oct. 21, 1913)
151. Goroshin, S. A., U.S.S.R. Pat. 25,724 (Mar. 31, 1932)
152. Gorter, E., and Seeder, W. A., *Kolloid-Z.,* **58,** 257 (1932)
153. Gortner, R. A., "Outlines of Biochemistry," New York, John Wiley & Sons, 1938
153a. Gould, S. P. and Whittier, E. O., *Milk Plant Monthly,* **27,** 46 (1938); *Natl. Butter and Cheese J.,* **29,** 34 (1938)
154. Gouse, M., and Leroy, C. E. H., French Pat. 767,110 (July 10, 1934)
155. Graenacher, C., U. S. Pat. 1,946,159 (Feb. 6, 1934); French Pat. 730,216
156. Grafe, K., *Z. angew. Chem.,* **49,** 303 (1936)
157. Granville and Davion, French Pat. 788,407 (Oct. 10, 1935)
158. Greenberg, D. M. and Greenberg, M. M., *J. Gen. Physiol.,* **16,** 559 (1933)
159. Greenhalgh, R., see Imperial Chem. Ind. Ltd., ref. 200

160. Groebe, F., and Reiner, S., *Gelatine Leim Klebstoffe,* **3,** 59 (1935)
161. Hacker, W., *Kunststoffe,* **22,** 229 (1932)
162. Hacker, W., *Kunststoffe,* **26,** 12 (1936)
162a. Hall, A. J., *Text. Col.,* **59,** 760 (1937)
163. Hansen, D. W., U. S. Pat. 2,047,961 (July 21, 1936)
164. Hansen, D. W., U. S. Pat. 2,074,332 (Mar. 23, 1937)
165. Hansen, D. W., U. S. Pat. 2,102,623 (Dec. 21, 1937)
166. Hansen, D. W., and Shildneck, P. R., Canadian Pat. 366,922 (June 22, 1937); Hansen, Canadian Pat. 366,923 (June 22, 1937)
167. Harry, R. G., *Paint Manuf.,* **7,** 214 (1937)
168. Hatschek, E., *Z. Kolloidchem.,* **8,** 34 (1911); **11,** 284 (1912); **12,** 238 (1913)
169. Haupt, K., *Chem. & Met. Eng.,* **39,** 319 (1932)
170. Haupt, K., *Kunststoffe,* **22,** 100 (1932)
171. Haupt, K., *Gelatine Leim Klebstoffe,* **2,** 55 (1934)
172. Haupt, K., and Wächtler, M., *Kunststoffe,* **15,** 129 (1925)
173. Heckel, H., U. S. Pat. 2,001,422 (May 14, 1935)
174. Hefti, F., Swiss Pat. 185,479 (Oct. 1, 1936)
175. Hemming, E., "Plastics and Molded Electrical Insulation," p. 110, New York, Chemical Catalog Co., Inc. (Reinhold Publishing Corp.) 1923
176. Henley, R. R., *J. Agric. Research,* **29,** 471 (1924)
177. Herald, A.-G., German Pat. 530,134 (Dec. 29, 1927)
178. Hill, H., Beckinsale, S., Slater, W. F., and Dunsheath, P., U. S. Pat. 2,085,978 (July 6, 1937)
179. Holland Zijde Mij, French Pat. 431,052
180. Haller, W., *Kolloid-Z.,* **56,** 170 (1931)
181. Holt, H. S., U. S. Pat. 1,978,533 (Oct. 30, 1934); 2,022,001 (Nov. 26, 1935)
182. Homberg, F., U. S. Pat. 1,786,488 (Dec. 30, 1930)
183. Homberg, F., and Landecker, M., U. S. Pat. 1,732,015; 1,732,016 (Oct. 15, 1929)
184. Hotta and Nakajimi, Japan Pat. 42,075 (Mar. 24, 1922)
185. Hull, S. M., U. S. Pat. 1,648,179 (Nov. 8, 1927)
186. Hull, S. M., U. S. Pat. 1,711,025 (Apr. 20, 1929)
187. Hutton, H. W., French Pat. 771,933 (Oct. 19, 1934)
188. I. G. Farbenind. A.-G., British Pat. 268,804 (Mar. 31, 1926)
189. I. G. Farbenind. A.-G., British Pat. 282,635 (Dec. 23, 1926); French Pat. 700,411 (Aug. 11, 1930)
190. I. G. Farbenind. A.-G., British Pat. 321,423 (June 30, 1928)
191. I. G. Farbenind. A.-G., British Pat. 279,863 (Feb. 26, 1929); French Pat. 643,030 (Oct. 26, 1927)
192. I. G. Farbenind. A.-G., French Pat. 701,744 (Sept. 6, 1930)
193. I. G. Farbenind. A.-G., French Pat. 719,891 (July 9, 1931)
194. I. G. Farbenind. A.-G., German Pat. 574,841 (Oct. 18, 1933) (R. Bauer and G. Mauthe); French Pat. 755,676 (Nov. 28, 1933); British Pat. 419,675 (Nov. 16, 1934); U. S. Pat. 1,979,601 (Nov. 6, 1934) (R. Bauer and G. Mauthe)
195. I. G. Farbenind. A.-G., British Pat. 411,058 (May 31, 1934); German Pat. 621,138 (Nov. 2, 1935)
196. I. G. Farbenind. A.-G., German Pat. 638,094 (Jan. 28, 1934); 639,175 (Nov. 30, 1936)
197. I. G. Farbenind. A.-G., French Pat. 784,283 (July 22, 1935)
198. I. G. Farbenind. A.-G., French Pat. 798,158 (May 11, 1936)
199. Imperial Chem. Ind. Ltd., French Pat. 785,788 (Aug. 19, 1935)
200. Imperial Chem. Ind. Ltd., and Greenhalgh, R., French Pat. 738,156 (June 4, 1932); British Pat. 379,739 (Sept. 5, 1932)

201. Internatl. Galalith Ges., German Pat. 368,942 (June 11, 1915)
202. Internatl. Galalith Ges., German Pat. 521,376 (Jan. 14, 1928)
203. Internatl. Galalith Ges., French Pat. 713,991 (Mar. 28, 1931); British Pat. 377,205 (July 20, 1932); German Pat. 588,177 (Nov. 14, 1933); U. S. Pat. 1,992,478 (Feb. 26, 1935) (to H. Dumont)
204. Internatl. Galalith Ges., British Pat. 380,677 (Sept. 22, 1932); 380,826 (Sept. 15, 1932); German Pat. 589,354 (Dec. 6, 1933) (to H. Dumont); U. S. Pat. 2,010,480 (Aug. 6, 1935) (to H. Dumont); French Pat. 714,569 (Mar. 27, 1931)
205. Internatl. Galalith Ges., French Pat. 735,874 (Apr. 23, 1932)
206. Internatl. Galalith Ges., German Pat. 593,224 (Feb. 23, 1934) (addn. to 588,-177)
207. Internatl. Galalith Ges., U. S. Pat. 1,992,478 (Feb. 26, 1934) (to H. Dumont)
208. Jaroslaw's Erste Glimmerwaren Fab., German Pat. 493,184 (June 18, 1926)
209. Jaroslaw's Erste Glimmerwaren Fab., British Pat. 272,947 (June 17, 1926); French Pat. 635,745 (June 10, 1927)
210. Jenkins, R. L., U. S. Pat. 2,010,517 (Aug. 6, 1935)
211. Johnson, E. H., U. S. Pat. 1,516,968 (Nov. 25, 1924)
212. Kagenek, v., German Pat. 281,541 (Nov. 12, 1913)
213. Kaiser and Gardels, U. S. Pat. 1,533,191 (Apr. 14, 1925)
214. Kasen, D., U. S. Pat. 2,045,471 (June 23, 1936)
215. Kathe, J., German Pat. 201,214 (July 14, 1905); 216,215 (July 20, 1905)
216. Kathe, J., British Pat. 17,953 (Aug. 7, 1907)
217. Katz, J. R., *Trans. Faraday Soc.*, **29**, 279 (1933)
218. Kausch, O., "Handbuch der kunstlichen plastischen Massen." München, Lehmanns, 1931; *Kunststoffe*, **27**, 149 (1937)
219. Kline, G. M., *U. S. Bureau of Standards Circ.* **411**, 24 (1936)
220. Koch, F. v., U. S. Pat. 2,051,779 (Aug. 18, 1936)
221. Koch, P. A., *Z. ges. Textil Ind.*, **39**, 306 (1936)
222. Kohner, A., British Pat. 102,375 (Nov. 15, 1917); U. S. Pat. 1,395,834 (Nov. 1, 1921)
223. Kraft-Phoenix Cheese Co., French Pat. 776,614 (Jan. 30, 1935); U. S. Pat. 1,968,991 (Aug. 7, 1934) (to F. H. Clickner)
224. Kraft-Phoenix Cheese Co., British Pat. 434,993 (Sept. 12, 1935)
225. Kratz, E. M., U. S. Pat. 1,847,656 (Mar. 1, 1932)
226. Kratz, E. M., U. S. Pat. 1,866,410 (July 5, 1932)
227. Kratz, E. M., U. S. Pat. 1,893,172 (Jan. 3, 1933)
228. Kratz, E. M., U. S. Pat. 1,976,027; 1,976,028 (Oct. 9, 1934)
229. Kratz, E. M., U. S. Pat. 2,027,829 (Jan. 14, 1936)
230. Kratz, E. M., and Heckel, H., U. S. Pat. 2,014,937 (Sept. 17, 1935)
231. Krische and Spitteler, U. S. Pat. 646,844 (Apr. 3, 1900); German Pat. 127,942
232. Kühl, H., German Pat. 280,648 (Aug. 21, 1913)
233. Kun, J., Hungarian Pat. 115,375 (Nov. 16, 1936)
234. Kutzelnigg, A., *Melliand Textilber.*, **18**, 201 (1937)
235. Labbe, L. L. T., U. S. Pat. 1,112,297 (Sept. 29, 1914)
236. Landecker, M., U. S. Pat. 1,725,805 (Aug. 27, 1929); 2,090,452 (Aug. 17, 1937); German Pat. 662,637 (July 19, 1938)
237. Landecker, M., German Pat. 641,531 (Feb. 5, 1937)
238. Lange, G., U. S. Pat. 2,103,163 (Dec. 21, 1937)
239. Larose, P., *Can. Textile J.* (Apr. 17, 1936)
240. Larose, P., *Am. Dyestuff Reptr.*, **26**, 584 (1937); *Can. J. Research*, **16B**, 61 (1938)
241. Laurin and Bidot, French Pat. 751,798 (Sept. 9, 1933); U. S. Pat. 1,969,932 (Aug. 14, 1934)

242. Lawrence, C. S., *Modern Plastics*, **14**, 30, 108 (1936); **15**, 16 (1937)
243. Lebreil, U. S. Pat. 1,189,841 (1916)
244. Lebreil and Desgeorge, French Pat. 361,896 (Nov. 9, 1905); German Pat. 240,584 (Dec. 12, 1909); Austrian Pat. 46,988; British Pat. 2,027 (1910); U. S. Pat. 1,023,022 (Apr. 9, 1912); 1,031,814 (1912)
245. Light, L., *British Plastics*, **3**, 291 (1931)
246. Lindener Eisen und Stahlwerke, German Pat. 304,994 (Sept. 2, 1916)
247. Ling, E. R., *J. Dairy Research*, **8**, 173 (1937)
248. Loomis, E. G., U. S. Pat. 2,075,735 (Mar. 30, 1937)
249. Lussaud and Montois, French Pat. 524,588
250. Magill, P. la F., U. S. Pat. 2,047,919 (July 14, 1936)
251. Maksorov, B. V. and Andrianov, K. A., *Plasticheskie Massui* No. **4**, 19 (1934); U.S.S.R. Pat. 30,276 (July 31, 1933)
252. Maksorov, B. V., and Andrianov, K. A., *Rev. gen. mat. plastiques*, **11**, 82, 115, 211 (1935)
253. Manfred, O., British Pat. 281,223 (Sept. 7, 1928) (continuation of 269,761)
254. Manfred, O., "Kolloid Chem. Tech.," 2d ed., 1931
255. Maries, F. G., British Pat. 169,405 (Dec. 7, 1920); U. S. Pat. 1,452,086 (Apr. 17, 1925)
256. Mestaniz, U. S. Pat. 353,697 (Dec. 7, 1886)
257. Miekeley *et al.*, German Pat. 642,398 (Mar. 3, 1937)
257a. Millar, German Pat. 88,225 (1895), quoted by Eggert, ref. 122a.
258. Mooser-Schliess, W., U. S. Pat. 1,317,721 (Oct. 7, 1919)
259. Morgan, G., *Chemistry and Industry*, **57**, 3 (1938)
260. Morgenstern, S., and Eggert, J., U. S. Pat. 2,109,432 (Feb. 22, 1938); 2,112,728 (Mar. 29, 1938)
261. Morin, French Pat. 588,441 (May 30, 1907)
262. Morrell, G., U. S. Pat. 2,022,895 (Dec. 3, 1935)
263. Morrell, G., U. S. Pat. 2,103,546 (Dec. 28, 1937)
264. Morrell, R. S., "Synthetic Resins and Allied Plastics," London, Oxford Univ. Press, 1937
265. Mougeot, G. F., *Rev. gen. mat. plastiques*, **14**, 35 (1938)
266. Müller and Mann, German Pat. 527,457 (Dec. 12, 1924)
266a. Murray, W. S., U. S. Pat 2,115,316 (Apr. 26, 1938)
267. Nakanishi, T., Japan Pat. 34,922 (Sept. 12, 1919)
268. Narici, E., *Svensk Papperstidn.*, **39**, 371; *Cellulosechem.*, **17**, 110 (1936)
269. Neubauer, C., German Pat. 523,591 (Oct. 16, 1926)
270. Neufeld, C. W., U. S. Pat. 2,096,684 (Oct. 19, 1937)
271. Nicol, H., *Fertilizer, Feeding Stuffs, and Farm Supplies J.*, **20**, 162 (1935)
272. Nielsen, H. C., U. S. Pat. 1,952,509 (Mar. 27, 1934)
273. N. V. Int. Kunsthoorn Ind., French Pat. 676,359 (June 7, 1929)
274. N. V. Int. Kunsthoorn Ind., German Pat. 514,771 (Aug. 3, 1929)
275. Obrist, J. and Manfred, O., *Rev. gen. mat. plastiques*, **3**, 755 (1927)
276. Obst., W., *Kunststoffe*, **22**, 86 (1932)
277. Olson, J., U. S. Pat. 1,705,780 (Mar. 19, 1929)
278. Osakeyhtio, S., British Pat. 326,208 (Dec. 4, 1928)
279. Oswald, O. and Co., German Pat. 610,557 (Mar. 12, 1935)
280. Ott, K., and Schüssler, H., German Pat. 575,927 (May 5, 1933); Canadian Pat. 284,967 (Nov. 20, 1928)
281. L'Oyonnaxienne, French Pat. 472,192 (Nov. 25, 1914)
282. L'Oyonnaxienne, French Pat. 565,338 (Jan. 24, 1924)
283. Pabst, F., French Pat. 652,615 (Apr. 12, 1928)
284. Patersen, A. E., U. S. Pat. 2,080,783 (May 18, 1937)
285. Pennenkamp, O., *Spinner und Weber*, **55**, No. 44, 24 (1937)

286. Pettie, W. F., U. S. Pat. 1,862,969 (June 14, 1932)
287. Pierson, W. C., U. S. Pat. 1,846,356 (Feb. 23, 1932)
288. Piperoux, R. P., Canadian Pat. 365,228 (Apr. 6, 1937)
289. Plauson, H., U. S. Pat. 1,395,729 (Nov. 1, 1921)
290. Plinatus, German Pat. 393,387 (Feb. 15, 1914)
291. Polden, D. C., U. S. Pat. 1,511,003 (Oct. 7, 1924)
292. Pontio, M., U. S. Pat. 1,293,191 (Feb. 4, 1919)
293. Potter, H. V., *J. Soc. Chem. Ind.*, **52**, No. 20, *Spec. Plastics Suppl.* 4 (1933)
294. Pozzi and Tandelli, French Pat. 386,011 (June 2, 1908)
295. Pufahl, *Kunststoffe*, **6**, 9 (1916)
296. Rankin, D. A. and Uhler, F. G., U. S. Pat. 2,030,226 (Feb. 11, 1936)
297. Raschig, F., U. S. Pat. 1,170,102 (Feb. 1, 1916)
298. Raschig, F., German Pat. 624,550 (Jan. 23, 1936)
299. Rath, H. and Burkhardt, R., *Klepzig's Textil Z.*, **40**, 292 (1937)
300. Rathke, E., *Chim. e ind., (Brazil)* **2**, 139 (1934); *Chem. Abst.*, **29**, 7526
301. Ray, G., *Milk Plant Monthly*, **25**, 38 (1936); *Lait*, **16**, 148 (1936)
302. Redecker, S. B., *U. S. Dept. of Commerce, Spec. Circ.* No. 356 (Oct. 30, 1931)
303. Redman, L. V., U. S. Pat. 1,732,533 (Oct. 22, 1929)
304. Reed, O. E., *Rept. of Chief of Bureau of Dairy Ind., U. S. Dept. of Agric.*, **25** (1937)
305. Reithoffer, J. N., German Pat. 147,994 (Feb. 19, 1901)
306. Reithoffer, J. N., German Pat. 141,309 (July 14, 1914)
307. Remise, L., French Pat. 791,551 (Dec 13, 1935)
308. Ripper, K., British Pat. 323,047 (Sept. 21, 1927); U. S. Pat. 1,952,941 (Mar. 27, 1934)
309. Ripper, K., German Pat. 559,105 (Sept 21, 1928); U. S. Pat. 1,952,941 (Mar. 27, 1934)
310. Rogers, L. A., "Fundamentals of Dairy Science," New York Chemical Catalog Co., Inc. (Reinhold Publishing Corp.), 1928
311. Roos, C. K., *Synthetic and Applied Finishes*, **3**, 156 (1933)
312. Rowell, H. W., "Technology of Plastics," London, Plastics Press, 1936
313. Satow, S., U. S. Pat. 1,245,978; 1,245,979; 1,245,983; 1,245,984 (Nov. 6, 1917)
314. Satow, S., U. S. Pat. 1,245,975; 1,245,976; 1,245,980 (Nov. 6, 1917); *Tech. Repts. Tohoku Imp. Univ. (Japan)* **2**, No. 2, (1921); **3**, No. 4 (1923); *J. Chem. Ind. (Japan)* **23**, 425, 811, 905 (1920)
315. Schade, A., and Schweitzer, E., U. S. Pat. 2,053,920 (Sept. 8, 1936)
316. Schäfer, J., Swiss Pat. 163,283 (Oct. 2, 1933)
317. Scheel, A., German Pat. 293,510 (Aug. 21, 1915)
318. Schlenker, H., *Papier-Fabr.*, **35**, Tech. Tl. 70, 80 (1937)
319. Schlinck, J., and Munchmeyer, G, U. S. Pat. 1,753,626 (Apr. 8, 1930)
320. Schmidt, F., German Pat. 510,693 (Sept 20, 1924)
321. Schmidt, F., British Pat. 281,993 (1927); French Pat. 636,219 (June 18, 1927); German Pat. 591,045 (Jan. 16, 1934); 597,349 (May 23, 1934)
322. Schmidt, F., U. S. Pat. 1,811,972 (June 30, 1931)
323. Schmidt, O., Seydel, K., and Meyer, B., German Pat. 482,930 (Aug. 28, 1926); U. S. Pat. 1,775,175 (Sept. 9, 1930)
324. Schmidt, O., and Meyer, E., German Pat. 540,723 (Dec. 4, 1928)
325. Scholer, C., *Am. Dyestuff Reptr.*, **26**, *Proc. Am. Assoc. Textile Chemists and Colorists*, 837, 840 (1937)
326. Schulte, W., U. S. Pat. 2,058,835 (Oct. 27, 1936)
327. Schultze, V., British Pat. 13,601 (June 10, 1909)
328. Schussler *et al.*, Canadian Pat. 284,967 (Nov. 20, 1928)
329. Schwarzbach, *Kunststoffe*, **7**, 69 (1917)
330. Schwarzberg and Noa, U. S. Pat. 864,388 (Aug. 27, 1907)

331. Shpital'nyi, A. S., Valeskevich, A. I., and Lyashch, R. W., *J. Applied Chem., (U.S.S.R.)* **10**, 1053 (1937) ; *Chem. Abst.*, **32**, 1462
332. Sichel, F., Komm. Ges., German Pat. 613,903 (May 28, 1935)
333. Simmons, W. H., *Ind. Chemist*, **6**, 206, 229, 297 (1930)
334. Sloane, C. E., U. S. Pat. 1,468,331 (Nov. 8, 1923)
335. Sly, C., U. S. Pat. 2,009,029 (July 23, 1935) ; 2,035,348 (Mar. 24, 1936)
336. Smidt, J. H., British Pat. 313,142 (June 8, 1928)
337. Smidt, J. H., British Pat. 352,100 (July 9, 1931)
337a. Smit, J., and van der Heide, B., *Nature*, No. 3571, p. 647 (1938)
338. Snell, H. S., U. S. Pat. 1,678,713 (July 31, 1928) ; 1,752,580 (Apr. 1, 1930)
339. Soc. anon. Franc. de Chim. Ind., French Pat. 425,204 (June 6, 1911)
340. Soc. anon. Franco du Ferodo, French Pat. 600,507 (Nov. 25, 1929)
341. Soc. d'Appl. et des Rech Sc. et Ind., German Pat. 542,969 (Apr. 27, 1929)
342. Soc. "La Cellaphane," British Pat. 446,383 (Apr. 29, 1936) ; 446,713 (Mav 5. 1936)
343. Soc. Comoy-Vincent, French Pat. 432,970 (Dec. 21, 1911)
344. Soc. pour l'ind. Chim. a Bale C. Graenacher, French Pat. 730,216 (Jan. 20, 1932) ; U. S. Pat. 1,946,159 (Feb. 6, 1934)
345. Soc. pour l'ind. Chim. a Bale C. Graenacher, Swiss Pat. 163,556 (Nov. 1, 1933) ; 182,395 (May 1, 1936) ; 189,027 (Apr. 16, 1937) German Pat. 654.393 (Dec. 18, 1937)
346. Sommer, K. H., British Pat. 470,707 (Aug. 19, 1937)
347. Sox. a. pn. Dunlop, French Pat. 757,432 (Dec. 26, 1933)
348. Spitteler, A., German Pat. 115,681 (July 15, 1899)
349. Sproxton, F., *J. Soc. Chem. Ind.*, **52**, *Spec. Plast. Suppl.*, 12 (1933) ; *Chem. Age (London)*, **28**, 428 (1933)
350. Stadt, H. M., U. S. Pat. 2,015,242 (Sept. 24, 1935)
351. Stand, C. J., and Briggs, R. M., U. S. Pat. 2,063,351 (Dec. 8, 1936)
352. Stark, C., *Gummi-Ztg.*, **45**, 1242 (1931) ; **47**, 79, 135 (1932)
353. Stark, C., *Rev. gen. mat. plastiques*, **8**, 15, 92, 145, 199, 267 (1932)
354. Stark, C., *Kunststoffe*, **22**, 220 (1932)
355. Staudinger, H., "Die Hochmolekularen Organischen Verbindungen; Kautschuk und Cellulose," Berlin, Julius Springer, 1932
356. Stephen, H., German Pat. 240,249 (Sept. 24, 1910) ; German Pat. 264,567 (Sept 11, 1912)
357. Stich, E., *Kunststoffe*, **5**, 158 (1915)
358. Stich, E., *Kunststoffe*, **5**, 185 (1915)
359. Stock, P., German Pat. 489,438 (Mar. 4, 1926)
360. Stockholms Benmjolefabriks Akt., French Pat. 757,892 (Jan. 5, 1934) ; 758,000 (Jan. 8, 1934)
361. Sturken, O., U. S. Pat. 2,053,850 (Sept. 8, 1936)
362. Sturken, O., and Woodruff, J. C., U. S. Pat. 2,040,033 (May 5, 1936)
363. Svedberg, T., Carpenter, L. M., and Carpenter, D. C., *J. Am. Chem. Soc.*, **52**, 241 (1930)
364. Tarsov, K. I., U.S.S.R. application 24,198 (Feb. 25, 1928) ; 29,211 (June 22, 1928)
365. Taylor, R. L., *Chem. & Met. Eng.*, **43**, 172 (1936)
366. Taylor, J. N., *U. S. Dept. of Commerce, Chem. Div.*, "Casein Solids"
367. Teillard-Chambon, M, see Wittouck, S., ref. 393
368. Telefunken Ges., German Pat. 618,822 (Sept. 16, 1935)
368a. *Textile World*, **88**, 78 (1938)
369. Thalmann, E., U. S. Pat. 2,090,434 (Aug. 17, 1937)
370. Thomas, F., French Pat. 450,635 (Nov. 15, 1912) ; U. S. Pat. 1,159,878 ; 1,159,879 (Nov. 9, 1915)

371. Todtenhaupt, J. C., *Z. ges. Textil Ind.*, **39**, 314 (1936)
371a. Todtenhaupt, J. C., German Pats. 170,051; 173,935; 183,317; 203,820
372. Tomasi, J. A., *Milk Plant Monthly*, **25**, 36 (1936)
373. Townsend, R. R. *U. S. Dept. of Commerce, Spec. Circ.* No. 347 (Oct. 19, 1931)
374. Tripet, R., French Pat. 808,497 (Feb. 8, 1937)
375. Tuckey, S. L., *Thesis*, Dept. Dairy Husbandry, Univ. Illinois, (1937)
376. Uhler, F. G., U. S. Pat. 2,063,134 (Dec. 8, 1936)
377. Ullmann, "Enzyklopädie der technischen Chemie" Vol. **5**, p. 579, "Galalith"
378. Ushakov and Eidlin, U.S.S.R. Pat. 23,613 (Oct. 31, 1931)
379. Van der Meulen, P. A., U. S. Pat. 2,069.787 (Feb. 9, 1937)
380. Vawter, W. E., U. S. Pat. 2,103,993 (Dec. 28, 1937)
381. Walsh, J. F., *Ind. Eng. Chem.*, **29**, 673 (1937)
382. Weber, E., German Pat. 538,065 (Dec. 2, 1928)
383. Weiss, R., German Pat. 313,881 (Apr. 29, 1917); British Pat. 115,439 (1918)
384. Wernicke, K., *Kunststoffe*, **2**, 181 (1912)
385. Wernicke, K., *Kunststoffe*, **2**, 205 (1912)
386. Weygang, C., British Pat. 111,171 (Nov. 22, 1916)
387. Whitmore, F. C., "Organic Chemistry," New York, D. van Nostrand Co., 1937
388. Whittaker, C. M., *J. Soc. Dyers Colourists*, **53**, 468 (1937)
389. Wiechmann, F. G., U. S. Pat. 1,080,188 (Dec. 2, 1913)
390. Weichmann, F. G., U. S. Pat. 1,061,346 (May 13, 1913); 1,135,340 (Apr. 13, 1915); 1,218,146 (Mar. 6, 1917)
391. Winkler, Austria Pat. 88,164 (Jan. 14, 1918); U. S. Pat. 932,527; 1,060,346
392. Wittouck, S., French Pat. 732,378 (Feb. 27, 1932)
393. Wittouck, S., British Pat. 383,069 (Nov. 10, 1932) (to Teillard-Chambon)
394. Wohak, G., Austria Pat. 150,629 (Sept. 10, 1937)
395. Wolf and Co., German Pat. 273,160 (1912)
396. Wolter, *Chem. Ztg.*, **33**, 11 (1909)
397. Woods, S. E., *U. S. Dept. of Commerce, Spec. Circ.* No. 364
398. Zeigmondy, A., German Pat. 515,376 (Dec. 4, 1926); British Pat. 298,815 (Dec. 22, 1927)
399. Zellmann, R., German Pat. 555,338 (Feb. 9, 1930); U. S. Pat. 1,922,069 (Aug. 8, 1933); 1,922,077 (Aug. 8, 1933)
400. Zillich, E., German Pat. 292,282 (Sept. 6, 1913)
401. Zillich, E., German Pat. 310,388 (Jan. 26, 1915)

Additional References Not Cited in Chapter 7

On Casein Plastics

Abramson, I. D. and Rybak, A. L., U.S.S.R. Pat. 36,012 (Apr. 30, 1934); *Chem. Abstr.*, **32**, 3525 (1938)
Molding powder of waste galalith ground and mixed with urea.

Akalit Kunsthornwerke A.-G., British Pat. 487,037 (June 14, 1938)
Soft plastic molded and then hardened in formaldehyde.

Anonymous, *British Plastics*, **2**, 16 (Sept. 1930)
Dip polish for hardened galalith, essentially hypochlorite solution.

Berstoff, H. Mach. G.m.b.H., German Pat. 496,821 (Apr. 28, 1930)
An extrusion press for kneading casein in making plastic.

Bumke, F. W., *Kunststoffe*, **28**, 60 (1938)
A review of the working and use of artificial horn.

Déribéré, M., *Rev. gen. mat. plastiques*, **11**, 311 (1935)
With Wood's light, ivory can be distinguished from its substitutes, such as casein plastic.

Deutsche Hydrierwerke A.-G., French Pat. 785,009 (July 31, 1935)
Alcohols of high molecular weight as plasticizers for casein.
Deutsche Hydrierwerke A.-G., French Pat. 792,989 (Jan. 14, 1936)
Organic complexes R-X-R'-(Y)n as plasticizers for casein.
Dodd, R. H., and Humphries, H. B. P., U. S. Pat. 1,143,625 (June 22, 1915)
Soybean protein dispersed and treated with alum and formaldehyde, and the coagulum separated, dried, and pressed.
Du Pont, E. I. de Nemours & Co., British Pat. 419,293 (Nov. 5, 1934)
Casein heated with polyhydric alcohol to form resinous mass.
Ellis, C., Jr., U. S. Pat. 2,125,776 (Aug. 2, 1938)
Horn powder with urea-formaldehyde for molding.
Francaise, La, French Pat. 817,593 (Sept. 6, 1937)
Casein in alkaline solution boiled with methylol urea or orthoxybenzyl alcohol to form a paste from which plastic masses may be formed.
Freeman, J., British Pat. 248,990 (July 7, 1925)
Method of gluing casein plastic material to wood.
Gallay, W., and Tapp, J. S., *Canadian J. Research,* **16B**, 345 (1938)
The effect of details of manufacture of rennet casein on the suitability of the product for making plastics was studied. A simple test of resistance to heat for determining the quality of rennet casein is described. The flow of moist casein during extrusion is represented by the equation $F = kP^n$, in which F is the flow, P the pressure, and n and k are constants characteristic of the casein and its moisture content. Plastic casein exhibits no yield point. Small increases in moisture content decrease the viscosity greatly. Decrease in pH, ash content, and lactose content of the casein all lower its viscosity. Up to 80°C. the temperature at which the casein is dried has little effect on viscosity. At pressures of 10,000 to 77,000 pounds per square inch viscosities as high as 1,700,000,000 poises were measured. The flow of plastic casein is streamline, which makes homogenization in the extrusion press difficult and often imperfect.
Grodzinski, P., *Kunststoffe,* **26**, 141 (1936)
Soybean resinous plastics in automobile construction.
Halen, S., *Kunststoffe,* **10**, 201 (1920). Review of earlier patents.
Hollandia, N. V., French Pat. 814,184 (June 17, 1937)
Machine and process for hardening casein plastic material so that it will not warp on drying, applicable particularly to buttons and small objects.
Houwink, R., "Elasticity, Plasticity and Structure of Matter," Cambridge Univ. Press, 1937
Fundamental treatment of elastic and plastic properties of casein, showing their dependence on water for plasticization.
I. G. Farbenind. A.-G., British Pat. 268,804 (Mar. 31, 1926)
Methylol ureas or urethanes as hardening agents incorporated cold and activated by hot molding.
Imperial Chem. Ind. Ltd., French Pat. 738,156 (June 4, 1932)
Condensation product of diphenyl propane and formaldehyde as a hardening agent activated by hot molding.
Jakova-Merturi, G., and Poggioli, J. A., French Pat. 635,637 (Mar. 20, 1928)
A chemical dip polish. Formolized casein or galalith, softened and rendered more water-resistant by treatment in boiling strong alkaline hypochlorite bath followed by treatment in hot mineral oil-sulfur-Cellophane bath.
Jakova-Merturi, G., French Pat. 623,650 (Oct. 25, 1926)
Describes a wet process for casein plastic material.
Lougee, E. F., *Modern Plastics,* **13**, No. 13, 54 (1936)
Soybean molding plastic in the automobile industry.
Max, A., French Pat. 810,741 (Mar. 27, 1937)
Casein and gelatin plastic hardened in 12 per cent alum or 20 per cent aqueous formaldehyde solution.
McKee, R. H., and Gould, S. P., *J. Agric. Research,* **57**, 125 (1938)
Fundamental research on the reaction between casein and formaldehyde.

Murray, W. S., U. S. Pat. 2,115,316 (Apr. 26, 1938); *Modern Plastics*, **15**, No. 12, 46 (1938)
Casein molding product made by treating skim milk with stearic or abietic acid and a chrom salt, to hydrolyze the lactose to aldehydes, which then react with the casein to harden it.
Parsons, J., U. S. Pat. 2,105,669 (Jan. 18, 1938)
Soft plastic rods chilled to permit working in automatic screw machines.
Rossiter, E. C., U. S. Pat. 2,097,895 (Nov. 2, 1937)
Dicyandiamide and formaldehyde form a series of resins miscible with proteins.
Sanfirov, G. I., U.S.S.R. Pat. 36,011 (Apr. 30, 1934); *Chem. Abstr.*, **32**, 3525
Galalith scrap ground and used with aniline oil and other ingredients to form a plastic material.
Schwartz, M. F., French Pat. 737,578 (Dec. 13, 1932)
Wet process for manufacturing casein plastics.
Schwarzberg, H., German Pat. 225,259 (Apr. 28, 1904); (Reissue Sept. 26, 1910)
Casein and starch with α-naphthol sulfonic acid for preparation of horn-like masses.
Schwarzberg, H., German Pat. 229,906 (Sept. 28, 1905); (Reissue Jan. 7, 1911)
Casein hardened with hexamethylenetetramine and aluminum acetate.
Soc. ind. mat. plastiques, British Pat. 276,542 (Dec. 1, 1926); French Pat. 637,157 (Nov. 2, 1926); Canadian Pat. 279,665 (Apr. 24, 1928).
Treatment of moist casein with formaldehyde in the presence of a swelling agent and retardant of the reaction with formaldehyde, and then heating under pressure.

On Synthetic Fibers from Casein

Akt. für Vermögensverwertung, German Pat. 659,655 (May 7, 1938)
An alkaline dispersion of casein containing 2 to 3 per cent carbon disulfide used for spinning fibers.
Cerbaro, E., *Chem. Abst.*, **32**, 3159 (1938)
Methylene blue test for detection of Lanital mixed with wool.
Diachenko, P., *Lait*, **18**, 233 (1938)
A dispersion of casein in ammonia gives a product superior to the Italian process using carbon disulfide.
Ferrari, C., *Ann. chim. applicata*, **27**, 482 (1937)
Lanital compared with other fibers from the point of view of analysis.
Genin, G., *Can. Dairy and Ice Cream J.*, **17**, 71 (1938)
A general article.
Kindermann, H., *Wollen und Leinen Ind.*, **58**, 225 (1938)
Fiber made from fish protein claimed to be superior to Lanital from casein.
Krenn, J., *Osterr. milchw. Ztg.*, **45**, 60 (1938)
A general article.
Mecheels, O., *Melliand Textilber.*, **19**, 579 (1938)
Fundamental molecular consideration of cellulose and protein as bearing on the probable characteristics of fibers produced from each.
Nippon Kari Kogyo K. K., French Pat. 827,992 (May 6, 1938); 828,075 (May 10, 1938)
Textile fibers made from soybean protein by a process similar to that for making Lanital.
N. V. Onderzoekingsinstutuut, French Pat. 823,962 (Jan. 29, 1938); French Pat. 825,841 (Mar. 15, 1938)
Alkaline dispersion of casein modified by addition of repressed formaldehyde and zinc salts. Makes stronger fiber.
Plail, J., *Melliand Textilber.*, **19**, 7 (1938)

Mixtures of casein and viscose described. The dry strength of Lanital is 3.2 g., wool 9.4 g.

Rath, H., Essig, A., and Abele, E., *Klepzig's Textil-Z.*, **41**, 463 (1938)
Qualitative test for Lanital in mixture with wool is possible through the formaldehyde loosely held, but the test cannot be made quantitative.

Roskin, E. S., *Legk. Prom.*, **16**, 88 (1937) ; *Chim. et Industrie*, **39**, 744
Comparison of Lanital with wool.

Söhngen, H., *Kunstseide und Zellwolle*, **20**, 78 (1938)
Review of the technical preparation of textile casein.

U. S. Department of Agriculture, "Committee Report on Development and Use of Rayon and Other Synthetic Fibers," Oct. 1938, p. 46
General description of the process and characteristics of Lanital. Italian production was 600,000 pounds in 1936, 3,500,000 pounds in 1937, and is estimated as 10,000,000 pounds in 1938. It is used chiefly in mixture with natural wool.

Vogt, G., *Kunstseide*, **19**, 241 (1937)
Outline of the progress of casein wool manufacture.

Wakelin, J., *Textile Colorist*, **60**, 515, 596 (1938)
Description of the use of proteins, especially glue and gelatin, with cellulose fibers to modify dyeing properties.

Chapter 8

Casein Glues

Frederick L. Browne and Don Brouse

Report has it that the craftsmen of ancient Egypt, Greece, Rome, and China used casein glue in their finest cabinet work, some of which is still to be seen in several museums. European woodworkers of the Middle Ages apparently understood the art of gluing with crude casein glues, an art that continued to flourish in a small way down to modern times. The manufacture of casein glue as a separate industry seems to have started in Switzerland and Germany in the early years of the nineteenth century. Patents on casein glues and sizes began to appear in the United States Patent Office as far back as the middle of the nineteenth century,[21, 31, 97, 108, 111, 121, 126, 127, 137, 152, 170, 187, 189, 193, 208, 225, 228] and between 1880 and 1890 casein began to be used in increasing quantities for coated paper;[218] but casein glue was practically unknown in this country before 1917.

When the United States was drawn into the World War, the need for water-resistant glue for the construction of military aircraft, which were then made chiefly of wood, suddenly aroused interest in casein glue and promptly led to a thriving industry in its manufacture. By the time that aircraft construction shifted largely to metal, casein glue was firmly established in other woodworking industries in which water-resistance proved advantageous. Subsequently glue from soybean meal,[23, 143, 150] which chemically is very similar to casein glue, largely displaced casein glue in the manufacture of softwood plywood, especially on the Pacific Coast; but the annual consumption of casein in glue remains several million pounds, although no statistics on the subject are available.

Preliminary Considerations

The noun "glue" without a qualifying adjective commonly means animal glue, because animal glue was much more widely used than any other kind for a very long time. The verb "to glue," however, indicates that a property rather than a kind of matter is the fundamental idea expressed by the word. It was to be expected, therefore, that the other substances that in modern times have partly replaced animal glue should likewise be called glues.

We may define a glue as a material obtainable in a viscous or a soft

plastic condition for purposes of application, and capable of changing subsequently to a jelly of high cohesion (the property underlying "jelly strength" and "tensile strength") and adhesion to surfaces to be joined. The appropriate consistency for obtaining a thick film on smooth surfaces that remains intact under pressure results usually from a condition of colloidal dispersion as a lyophilic sol. The term "cement," sometimes used as a synonym for glue, might properly be reserved for those materials, applied in a condition of plastic, lyophobic suspension having a high yield value, that set to hard, stony masses largely through the formation of interlocking crystals or crystals embedded in an amorphous matrix. Typical cements may be poor glues and are often weak in tension, though strong in compression.

Classification of Glues

Glues may be classified conveniently according to the manner in which gelation is brought about. A scheme based on the physical properties of the glue instead of on the raw material from which it is made seems logical, in view of the fact that it is the properties that control the usefulness of the glue in industry rather than the materials from which it is derived.

1. *Glues that gel by cooling of a lyophilic sol.*

Hide glue and bone glue are typical of this class. They swell in cold water and melt on warming. When the temperature falls again they gel, and the strength of the resulting jelly increases as it dries.

2. *Glues that gel by the heating of a lyophilic sol or of a resin.*

Albumin glues of the more common type are heated to temperatures usually in the neighborhood of 100°C. to bring about gelation. Another type of albumin glue belongs to Class 3. The hot-press resin glues require heating to complete the condensation reaction whereby the final insoluble and infusible resin is formed.

3. *Glues that gel without temperature change,* as a result of a chemical reaction taking place slowly in a solution or in a sol.

The water-resistant casein glues belong to this class. The casein may be dissolved first as sodium caseinate in a solution containing calcium hydroxide. Calcium caseinate then forms by reaction between the sodium compound and the lime, and being stable in the gel form only, under the conditions prevailing, the glue slowly forms a jelly. Both animal and blood glues of this type can be prepared by the introduction of compounds whose reaction liberates formaldehyde, which in turn combines with the protein to form a compound capable of gelation.[42, 149]

4. *Glues that gel through evaporation of solvent.*

a. Glues using water as the solvent. The non-water-resistant casein glues, vegetable (starch) glues, liquid glues, mucilage, and sodium silicate are dispersions or solutions in water that gel as they dry.

b. Glues using non-aqueous solvents. Nitrocellulose glues are made with non-aqueous solvents, evaporation of which leaves a jelly.

5. *Glues in which gelation is brought about without use of a solvent.* Casein, albumin, and resin glues used by dry gluing processes fall in this class. The glue in a suitable solvent may be applied to one or both of the surfaces to be joined and the solvent allowed to evaporate; or tissue paper may be impregnated with the glue in that way and placed between the surfaces;[90, 206, 216] or the glue in granular form may be distributed between the surfaces,[56, 57] the surfaces being then brought together and pressed in a hot press to melt the glue and, in the case of resin glues, to complete the condensation reaction.[14, 95, 147, 167, 205, 227]

In considering a system of classification based upon the manner in which gelation is brought about, it must be remembered that in a given glue more than one factor may, and in fact generally does, affect gelation. Thus the change of a high-grade hide glue from the sol to the gel condition is affected to some extent by loss of water through evaporation or absorption by the wood, but the outstanding factor that must be considered in using it is the temperature effect. It therefore belongs in Class 1 rather than in Class 4*a*. By suitable treatment, however, this same hide glue might be changed into a liquid glue in which gelation depends upon loss of moisture, placing it in Class 4*a*. Again, by incorporating a formaldehyde compound, a glue may be made whose gelation depends primarily upon a chemical reaction, but in which both temperature and evaporation effects must be kept in mind. Such a glue belongs rightly in Class 3. The classes in the above scheme are not separated by sharp lines of demarcation, but they represent the several factors upon which gelation may depend and whose predominating influence governs the use of a glue in industry.

Water-resistant Glues

Most of the glues used in woodworking are stronger than the shearing strength of wood, and when properly applied are capable of giving joints in which the failure produced by sufficiently high shearing stress takes place chiefly in the wood rather than in the glue line, provided that the glue is reasonably dry. As the joints take up moisture, however, both wood and glue become weaker. Ordinary animal glue, vegetable glue, and some types of casein glue are more seriously weakened by absorption of moisture than is wood, so much so that the joints finally fall apart under the stress of the swelling of the wood, especially in the case of a joint in plywood. Water-resistant animal glue, casein glue, or blood albumin glue, though weakened more seriously than wood, remain strong enough not only to hold the joint together but to retain some reasonable fraction, say 25 per cent or more, of the original strength of the joint

when dry. The extent to which the wood itself is weakened by absorption of water may be illustrated by the following data for the strength

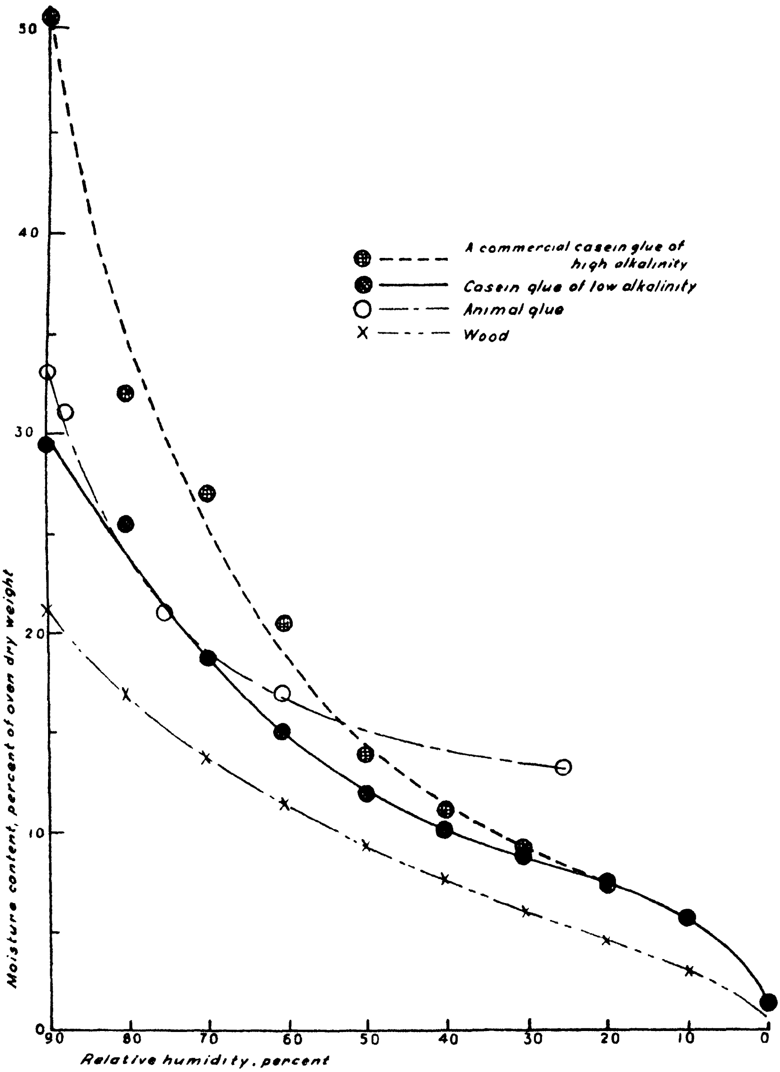


Figure 29.—The moisture content of casein glue, animal glue, and wood in equilibrium with atmospheres of varying relative humidity.

of sugar maple in shear parallel to the grain: 2,330 pounds per square inch when air dry and 1,460 when green.[153]

The term "waterproof" should be used with great caution because few

materials are unaffected by water. Many casein glues were formerly offered by their manufacturers as "waterproof" glues, a designation that had to be altered when resin glues of still greater resistance to water appeared on the market.

Water-resistance is not so much a question of the amount of water absorbed by the glue jelly, as it is of the extent to which the absorption of moisture weakens the jelly. Figure 29 shows hygroscopicity curves for jellies of casein glues of high and low alkalinity,[41] of animal glue,[9] and of wood[94, p. 191] at 70°F. Animal glue, which has little water-resistance, absorbs less moisture in damp atmospheres than highly alkaline but water-resistant casein glue. The strength of the jelly of animal glue in tension, however, falls from 20,000 pounds per square inch at 13 per cent moisture content to 8,000 pounds at 24 per cent and 500 pounds at 30 per cent,[9] whereas the jelly of casein glue at 30 to 50 per cent moisture content is nearly as strong as it is when dry, at least until hydrolysis or decay of the casein sets in.[41] The hygroscopicity curves for vegetable glue and blood glue are very similar to that of the casein glue of low alkalinity in Figure 29, yet the jelly of vegetable glue loses practically all strength at 30 per cent moisture content while that of blood glue remains for some time almost unaffected.

It should be noted that the property of water-resistance is due to the physical state of the glue jelly rather than to the nature of the protein, carbohydrate, or resin forming the jelly. Casein glues of Classes 3 and 5 are water-resistant but those of Class 4*a* are not. Ordinary animal glue lacks water-resistance, but that property can be imparted by suitable treatment. Water-resistance depends upon bringing about an *irreversible* gelation. In the case of water-resistant animal glue and some blood glues, irreversible gelation is effected with a compound of formaldehyde; with blood glue and some casein glues it is accomplished through heat coagulation of the proteins in a hot press; and with cold-press casein glues by coagulation with lime. In the case of hot-press resin glues irreversibility is achieved by completing the resinification reaction in the hot press.

It should also be noted that the layer of water-resistant glue in a joint is not necessarily less permeable to moisture than a similar layer of non-water-resistant glue. Moisture moves through plywood glued with water-resistant casein and blood glues about as readily as it does through similar plywood glued with animal or vegetable glue.[125] On the other hand, some of the resin glues make plywood through which moisture moves very slowly indeed.

The Nature of Adhesion

The mechanism of adhesion apparently seems mysterious to many persons,[6, 10] yet there is no reason for its being so because it is closely

related to everyday properties of matter that we all accept without question.[38] Hardy,[119] encountering adhesion as a limiting case in a study of lubrication, identified adhesion with the force involved in the wetting of a solid by a liquid and in adsorption. Fundamentally it is the same force responsible for the liquid and solid states of matter, for solutions, and for the failure of real gases to comply exactly with the law of ideal gases. In other words it is the correction factor a in van der Waals' equation of state. Molecules of all kinds, if brought closely enough together, exercise an attraction upon one another. Where the necessary proximity can be established, adhesion is the general expectation and it is lack of adhesion that should call for explanation.

The problem of establishing adhesion between the surfaces of two solids is one of bringing them together closely enough for the forces of adhesion to come into play, for the range of action of these forces is of the order of 3×10^{-8} centimeter. In practice there are two difficulties. In the first place only exceptionally smooth and accurately matched solid surfaces can be brought so close together. From the point of view of such infinitesimal distances, even what we ordinarily consider highly polished surfaces are exceedingly rough and far from level. In the second place the surface of each of the solids as a rule is already covered by an adsorbed layer of air, moisture, or whatever the solid may have previously been in contact with. The adsorbed materials must be displaced before the solids can be brought together intimately.

Since liquids can readily follow the contours of a solid surface, it is much easier to establish adhesion between a liquid and a solid than between two solids. All practical technics of gluing solids together, therefore, make use of a liquid that wets and establishes contact with each of them and then solidifies in place, forming a rigid layer completely filling the space between the solids joined.[157]

Adhesion through intermolecular attraction was designated specific adhesion by McBain and his students.[144, 157, 158] It is the only kind of adhesion available for attachment to smooth, level surfaces of nonporous solids. At the surfaces of porous solids, however, another kind of adhesion may become operative, which McBain termed mechanical adhesion. A gross example of mechanical adhesion is the grip of plaster on lath. Because mechanical adhesion is so readily visualized and specific adhesion has been so often treated as a mystery, it is commonly assumed that all adhesion at the surfaces of porous solids is strictly mechanical. Generally speaking, however, porous solids are composed of much the same sort of matter as nonporous solids, and they are capable of exerting the same sort of specific adhesion, provided only that the necessary intimate contact is made with their surfaces. The porous solids, therefore, differ from the nonporous solids in that both kinds of adhesion rather than only one may be operative at their surfaces.

McBain's statement that adhesion to wood is purely mechanical[59, 157, 158] has been widely misinterpreted to mean that the adhesion is confined to mechanical grip by embedding in those cavities of the wood structure that can be seen with the aid of a microscope. The glues commonly used in woodworking, however, adhere firmly to the walls of those wood cells into which they penetrate; and mechanical adhesion is attainable under the ordinary conditions of gluing only when the glue, while in the liquid condition, has the property of wetting wood.[40, 43, 107, 214] McBain held, however, that adhesion to the walls of the wood cells is likewise mechanical rather than specific. Mechanical adhesion in "pores" that are too small to be resolved in a microscope should properly be called ultramicroscopic mechanical adhesion, and should be clearly distinguished from the layman's concept of mechanical adhesion.

The assumption of ultramicroscopic mechanical adhesion serves no useful purpose. An arbitrary dividing line between smooth and rough surfaces must be drawn somewhere because all surfaces are rough and all solids porous if the unit of measurement is taken small enough. Access to pores of ultramicroscopic size can be gained readily only by liquids that wet the solid and are therefore drawn to it closely enough to be within the range of action of specific adhesion. If the liquids then solidify in place, specific adhesion remains operative, at least in those cases, like the formation of a colloidal jelly, in which solidification takes place without sudden change in shape or volume.

General Description of Casein Glues

From the point of view of marketing methods casein glues may be classified as (a) prepared glues, (b) wet-mix glues.

Prepared glues are sold in the form of dry powders containing all necessary ingredients except water. The user adds the powder to water at room temperature in the proper proportions, usually about 2 parts of water to 1 of glue by weight, employing a mechanical mixer. Most of them can be mixed ready for application even in large batches in less than 30 minutes. Once mixed, the glue must generally be used within a working day or less. Some casein glues used in fields other than woodworking must be warmed to bring them into solution and they may not be subject to the restriction of use within the working day.

Wet-mix glues are made as required for use from ground casein, water, and the additional chemicals called for by the formula.

Both prepared and wet-mix glues are used by the woodworking industries in large quantities. Each type has advantages. Wet-mix glues permit the use of certain desirable ingredients that cannot very successfully be incorporated in prepared glues. They make it possible to add the components in the most advantageous order, a matter of importance in some formulas. The casein can be allowed to swell thoroughly in water

before adding the other reagents so as to bring it into solution more rapidly and uniformly. With wet-mix glues the formula can be varied at will to meet special requirements, and in general a smaller proportion of alkali can be used in making the glue. Possibly the most important reason for the wide use of wet-mix glues is the fact that the formulas commonly used (of which formulas **K** and **L** are typical) require the addition of slightly more water per pound of dry ingredient and involve very inexpensive materials, so that the cost per square foot of glue line may be less than that of prepared casein glues.

Prepared glues, on the other hand, require the purchase and storage of but one material instead of three or more. In mixing prepared glue there are two components to measure out instead of four or more, and it is possible to measure them by volume, dispensing with the use of a balance altogether, although such practice is not recommended. When a wet-mix glue is used the ingredients must be carefully weighed by a trustworthy operator if uniform results are to be obtained. Such a man is not always to be found in charge of the glue room of woodworking plants. The manufacturer of prepared casein glue takes the responsibility of seeing that the casein and other ingredients of the glue are of proper quality for the purpose and incorporated in the correct proportions. Since technical caseins vary widely enough to require variations in the formula in order to keep the consistency and working life of the glue uniform, and since there are several qualities of lime and of sodium silicate on the market, this service performed by the glue manufacturer is an important advantage in favor of prepared glue.

The casein glues patented in the United States before 1901 were all of the wet-mix type, were often very difficult and tedious to prepare, and cannot now be considered water-resistant.[110, 121, 187, 193] The first step toward prepared glues was the manufacture of "soluble casein," that is, the metallic or ammonium caseinates.[15, 30, 71, 116, 135, 136, 152, 225] Presumably the soluble caseins were intended primarily for sizes and paper coatings and when used for glues were to be mixed with other ingredients besides water.

The use of lime water was first mentioned in a patent in 1876[193] and lime in 1897[97] and 1899[110] but the fact that no patent claims invention of the use of lime in casein glue suggests that it was taken from prior art. Use of magnesia or other "infusible oxide" was patented in 1902.[204] Other agents intended to develop water-resistance that are revealed in early patents are: formaldehyde,[73, 109, 197, 211] hexamethylenetetramine,[79] tannic acid,[15, 136] drying oils,[212] hydraulic cement,[130] and preliminary heating of the dry casein;[61, 122] but none of these has proved practicable.

The first prepared, water-resistant glue appeared in a patent to Hall[117] in 1901: a patent that expired just as a substantial market for the product developed for the first time. Subsequent patents for prepared casein

glues follow essentially the same basic chemistry but make use of different reagents. Apparently the possibility of manufacturing casein glue in a paste form, analogous to the casein paste paints, has not yet been explored, although a neutral casein glue (without water-resistance) has been patented.[217]

Chemistry of Casein Glue

The voluminous literature concerning the chemistry of casein must be applied cautiously to the problem of casein glue for the reason that the concentrations involved are very unusual and the casein itself is undergoing profound alterations. There is present usually only 3 or 4 grams of water per gram of casein, the water contains 5 or 10 per cent of dissolved alkalies and salts and has a pH exceeding 12. In solutions of such high alkalinity the casein hydrolyzes, as is evidenced by the strong odor of ammonia, so that by the time the glue has set to a jelly, much if not all of the casein has been split into its primary cleavage products, the composition of which is even less well understood than that of casein. The condition in casein glue thus bears a strong analogy to animal glue in which hydrolysis of the glue continues as long as it is kept at the temperatures necessary for working. In both cases the ability of the glue to form a strong jelly decreases as hydrolysis goes on, at least beyond a certain point.

For the purpose of discussing their chemical composition it is desirable to divide casein glues into (*a*) Class 4*a* glues forming reversible jellies by evaporation and (*b*) Class 3 glues that gel spontaneously, forming irreversible jellies. The former are used to a certain extent at the present time for perhaps a large number of practical purposes, but the total amount consumed is undoubtedly much less than of the latter. They are not very highly water-resistant. The woodworking industries, which consume the great bulk of the casein glue produced, generally use glues of Class 3. For the most part they are water-resistant, although for certain purposes the water-resistance may be sacrificed very largely in order to secure some other desirable property.

Glues Giving Reversible Jellies

Solutions of casein in alkalies containing enough of the protein to give a suitable viscosity can be used as glues. By varying the proportion of casein to water or by changing the ratio of alkali to casein at the same water content, the viscosity can be altered at will from a thin, watery solution to the consistency of a very thick animal glue or of molasses. This makes it possible to prepare a casein glue that will have a given viscosity with the use of a comparatively small concentration of the protein, or one containing a large proportion of solids without being too thick to flow readily, as may be demanded by the conditions under which

it is to be used. Casein glues of this kind are capable of giving glued joints comparing favorably in strength with those made with animal glue, but they are not water-resistant, at least as the woodworker uses that term.

The alkali most commonly employed, although it may be introduced indirectly, is sodium hydroxide.[12, 93] Ammonium hydroxide may also be used[12] and has certain advantages when it is desired either to obtain a high viscosity with as small a concentration of casein as possible or to minimize hydrolysis of the protein. If we start with the same or similar caseins, glues of very much the same consistency can be made by any of the following formulas:

A	{ { Casein	100 grams	
	{ Water	300	
	Sodium hydroxide	8	(0.2 gram equivalent)
B	{ { Casein	100 grams	
	{ Water	600	
	Sodium hydroxide	4	(0.1 gram equivalent)
C	{ { Casein	100 grams	
	{ Water	600	
	Ammonium hydroxide	13 cc.	(containing 28–29 per cent NH_3) (0.2 gram equivalent)

In these and the remaining formulas in this chapter, the brackets are used to indicate the order in which the components are mixed. Thus in formula **A** the casein is first allowed to swell by soaking it in the water and the sodium hydroxide added later. The best practice is to use a small portion of the 300 grams of water indicated for dissolving the sodium hydroxide before adding it to the casein pulp. The proportions of the ingredients stated are suitable for general woodworking purposes but are subject to variation within limits that will be brought out in further discussion.

It should be noted that formulas **B** and **C** contain only half as much casein per unit of volume as formula **A** although the consistency is roughly the same. In applying glues, the spreading rate, that is, the area of joint surface covered by a unit quantity of glue solution, depends very largely upon the consistency of the glue; hence it is evident that formula **A** requires more casein per unit area of joint surface than formulas **B** or **C**, other conditions remaining the same. Since the consistency of the glue in certain cases may have to be kept within definite limits determined by the mechanical operations of the process, this relation between consistency and the amount or kind of alkali used may often be used to advantage to control the quantity of casein (or of prepared glue) required to glue a given area of joint surface.

In making these glues, the best procedure is to weigh the casein into

the mixing vessel, add the required amount of water, stir until each particle of casein has become moist, and then let it stand until the casein has completely swollen. The time required for this varies somewhat with the fineness of grinding of the casein and the temperature at which the curd was dried, but with commercial casein of good quality it should not exceed 5 or 10 minutes. The alkali is then added and the mixture stirred thoroughly until the casein is dissolved. With formula **A** solution is generally complete in less than 10 minutes, but with formulas **B** and **C** one-half to one hour may be necessary. If the mixture is warmed slightly or if warm water is used to soak the casein, solution may be brought about within a few minutes.

Where it is not convenient to heat the mixture, a glue that is essentially formula **C** may be made rapidly as follows:

D	Casein	100 grams	
	Water	600	
	Sodium hydroxide	8	(0.2 gram equivalent)
	Ammonium chloride	10.7	(0.2 gram equivalent)

After soaking the casein in the water, it is brought into solution quickly by adding sodium hydroxide. The solution is then very thin. On adding the ammonium chloride, which reacts promptly with the sodium hydroxide to form ammonium hydroxide and sodium chloride, the solution thickens rapidly and acquires a viscosity suitable for use as a glue.

Formulas **A**, **B**, **C**, and **D** are suitable for wet-mix glues only. The hygroscopic character of sodium hydroxide makes it difficult to store a casein glue containing it without danger of deterioration. It is an easy matter, however, to introduce the alkali in an indirect manner so that the casein can be mixed with all of the necessary ingredients except water, giving a dry powder that is easily handled and stored, requiring only the addition of water to prepare the glue for use. One way to do this is to replace the sodium hydroxide in formula **A** or **B** with chemically equivalent amounts of calcium hydroxide and a substance that, when dissolved in water, reacts with the calcium hydroxide to form sodium hydroxide. Any convenient sodium salt of an acid whose calcium salt is relatively insoluble may be used for the purpose, provided that it is not hygroscopic and does not react with the lime or the casein as long as the mixture is kept dry. A general formula for glues similar in most important respects to formula **A** may be written as follows:

	Casein	100 grams	
E	Calcium hydroxide	7.4	(0.2 gram equivalent)
	Sodium X	0.2 gram equivalent	

Here X is an anion with which calcium ions form a precipitate of CaX_2. Sodium X may be, for example: sodium carbonate, sodium oxalate, sodium tartrate, sodium citrate, sodium salicylate, sodium phos-

phate, sodium fluoride, sodium sulfite, sodium arsenate, sodium arsenite, sodium stannate, etc. Similarly the potassium salts, or salts of any of the alkali metals could be used in place of the sodium salts. To prepare the glue for application, the dry powder is added to water in such proportion as to give 300 grams of water per 100 grams of casein.

Glues of formula **E** differ in one important respect from those of **A**, namely, when mixed with water ready for use they remain fluid only for 15 to 20 hours, after which they form irreversible jellies. Apparently the precipitate of CaX_2 reacts in part with the sodium caseinate to form enough calcium caseinate to bring about gelation, or else sodium caseinate on adsorbing the calcium compound acquires the property of gelation. The relation between the casein and CaX_2 is undoubtedly similar to that of the casein and the calcium phosphate in milk. Glues of formula **E** may have a certain degree of water-resistance, but not enough to classify them as water-resistant from the woodworker's point of view.

Formula **E** must not be confused with formula **H**. In formula **E** the amount of calcium hydroxide does not exceed chemical equivalence to the amount of sodium X. When the glue is added to water the calcium hydroxide is changed, presumably completely, to calcium X, leaving no free lime in the mixture.

Another method of introducing sodium hydroxide into prepared casein glue consists in the addition of sodium salts that are stable when dry but hydrolyze freely when dissolved in water. When the mixture is added to water, the salt on dissolving hydrolyzes to an extent governed by the dissociation constant of the resulting weak acid. The casein reacts with the sodium hydroxide liberated to form sodium caseinate, whereupon further hydrolysis of the salt takes place. Equilibrium is finally reached when such a distribution of the sodium hydroxide between the casein and the weak acid is reached that both are in equilibrium with a solution of the same pH. The amount of sodium caseinate is greater, the larger the quantity of the salt added and the smaller the dissociation constant of the acid. Among the salts that have been suggested for the purpose are: borax,[12, 70, 71, 97] sodium carbonate,[12, 97] sodium phosphate,[12, 109, 118] sodium fluoride,[74, 129, 180, 188] sodium hyposulfite,[109] sodium arsenate,[15] sodium tungstate,[52] sodium aluminate,[180] sodium resinate,[12, 98, 121] sodium salts of weak organic acids,[136,180] and, for wet-mix glues, sodium silicate.[19, 52, 64, 97, 131] The proportion of hydrolyzable salts required to bring the casein into solution varies widely. For example, glues were made in accordance with the following formula:

F	Casein	100 grams
	Water	600
	Hydrolyzable salt	y

in which y represents the least quantity of the hydrolyzable salt found necessary to bring the casein into solution satisfactorily. The value of y for various salts is given in Table 20.

TABLE 20.—Proportion of Hydrolyzable Salt Required for Formula **F**

Hydrolyzable salt	y in Grams	y in Gram equivalents	Dissociation constant of resulting acid
Borax, $Na_2B_4O_7 \cdot 10\ H_2O$	14.7	0.077	5×10^{-10}
Trisodium phosphate, $Na_3PO_4 \cdot 12\ H_2O$	12.3	0 097	
Sodium stannate, $Na_2SnO_3 \cdot 3\ H_2O$	20	0.15	4×10^{-10}
Sodium carbonate, Na_2CO_3	16	0.30	3×10^{-7}
Sodium arsenate, $Na_3AsO_4 \cdot 12\ H_2O$	30	0.21	5×10^{-3}
Sodium fluoride, NaF	64	1.5	

The minimum amount of sodium hydroxide required to dissolve the same casein under similar conditions was about 3.95 grams (0.099 gram equivalent). With such small proportions of alkali the casein dissolves rather slowly at room temperature, but if the mixture is warmed to 60°C. solution can be brought about in a few minutes. In the case of sodium fluoride it is necessary to apply heat to dissolve the casein at all.

Formula **F** is written as a wet-mix glue, that is, the casein is first soaked in water before adding the sodium salt. Prepared glues can be made by mixing the casein with any of the salts mentioned. It is advisable, however, to use much larger proportions of the hydrolyzable salts if the glue is to be mixed at room temperature. It is also necessary to grind the casein to a finer degree of subdivision than most commercial caseins. On the other hand, too fine grinding is to be avoided because it involves danger of formation of lumps of partially dissolved casein when the glue is mixed with water. Casein that passes 60- but is retained on 80-mesh sieves is suitable for prepared glues.

The hydrolyzable salts have another important advantage over sodium hydroxide as ingredients of a casein glue in that the properties of the glue are not altered greatly by small changes in the proportion of salt. With formula **B**, for example, a slight increase in the amount of sodium hydroxide reduces the viscosity of the glue enormously, while there is danger of rather rapid hydrolysis of the casein if much more sodium hydroxide is added than is called for in formula **A**. Because of the buffer action of the hydrolyzable salts, however, the proportion used can be varied within wide limits without endangering hydrolysis or altering the viscosity too much.

The marked variation in viscosity of dilute solutions of casein in alkalies as the pH of the solution changes is shown in Figure 5. The viscosity reaches a sharp maximum near pH 12. Zoller[229] found that in 9 per cent solutions of casein the maximum occurs at pH 9.2, beyond which point the viscosity in solutions of alkali metal hydroxides falls off very rapidly. The difference in the amount of water required to produce glue of given consistency according to formulas **A** and **B** is thereby explained. At a given pH glues made with sodium hydroxide require somewhat more water than glues made with potassium hydroxide. When ammonium hydroxide is used, however, a still higher maximum is attained at pH 9.2, after which the viscosity remains nearly at the maximum as the pH increases still further. The high water requirement of formulas **C** and **D** finds explanation in that fact. Zoller considered that, inasmuch as evolution of ammonia is one of the first steps in hydrolysis of casein, ammonium hydroxide represses the alkaline hydrolysis of casein by mass action and thereby prevents the decline in viscosity beyond pH 9.2 which is characteristic of solutions in alkali metal hydroxides. In the latter case he attributed the decline in viscosity to hydrolysis of the casein.

Zoller likewise found a sharp maximum in viscosity at pH 9.2 when casein is dissolved in most of the hydrolyzable salts, making solutions containing 9 per cent of casein. With borax, however, he found the maximum at pH 8.1. Zoller suggested that boric acid forms a complex with the polyhydroxyl groups of the casein molecule, like the complex known to be formed between boric acid and glycerol or various sugars, the effect of which is an increased dissociation of the boric acid. At the pH of maximum viscosity, solutions in trisodium phosphate are much more viscous and solutions in sodium carbonate, sodium silicate, or sodium arsenate less viscous than solutions in borax or sodium fluoride.

In selecting alkalies or hydrolyzable salts and working out the formulas for casein glues, these viscosity relations should be kept in mind. A thick glue that will remain in place between the surfaces to be joined in spite of a high pressure can be obtained either by decreasing the proportion of water or by selecting the alkali-producing substance and using it in the right proportion to obtain maximum viscosity with high water content. On the other hand, if a glue is needed that will have a maximum fluidity and yet introduce as much casein and as little water as possible into the joint, it is best to work with an alkaline substance and in a region of pH in which the viscosity is at a minimum and hydrolysis is not yet too rapid.

Casein glues of the reversible jelly type remain in a workable condition for a relatively long time after they have once been mixed. They cannot be kept safely in the liquid condition indefinitely, however, be-

cause they are subject to two disintegrating influences, bacterial decomposition and hydrolysis. The former can be minimized by adding antiseptics[106] such as beta naphthol,[35] thymol,[124] nitrobenzene,[124] phenol,[142] sodium orthophenyl phenate,[8] and proprietary preservatives,[12, 124] but the latter offers greater difficulties. The rate of hydrolysis increases with alkalinity and is rather rapid in those ranges of pH that, for other considerations such as viscosity, are generally preferred. If the alkalinity can be kept below pH 10, however, casein glues can be kept in a workable condition for many days. The first effect of hydrolysis is a marked decrease in viscosity, but adhesive qualities are not lost until hydrolysis has reached a fairly advanced stage.

Glues Giving Irreversible Jellies

The casein glues giving reversible jellies are of relatively minor importance industrially, though they do find some uses[12, 13, 142, 145, 219] where water-resistance is not important. Discussion of glues giving reversible jellies, however, forms a logical introduction to the more important glues yielding irreversible jellies.

Although patents continue to appear in which formaldehyde,[120] hexamethylenetetramine,[120] paraformaldehyde,[169] and acetaldehyde[11, 25] are used to make the jelly irreversible, none of these reagents has proved practicable in woodworking glues. Treatment of plywood panels with formaldehyde gas or solution shortly after gluing accomplishes the desired result[186] but is of very limited applicability. In practically all commercial casein glues that are water-resistant, the jelly is rendered irreversible by changing the alkali caseinate to a caseinate of a heavy metal or alkaline earth metal, usually to calcium caseinate.

Calcium caseinate is generally preferred for commercial water-resistant glues, partly because lime is cheaper than any other hydroxide and still more because it happens to have just the right solubility in water. Calcium chloride added to a solution of casein in sodium hydroxide precipitates the protein instantly as a curd, undoubtedly because the highly soluble salts react so rapidly that the formation of a jelly cannot take place. The limited solubility of calcium hydroxide makes the calcium available for reaction much more slowly so that gelation requires some time. If calcium salts less soluble than the hydroxide but not too slightly soluble are used, calcium tartrate, for example, gelation under the same conditions of temperature, concentration, and pH takes place but requires a longer time. Irreversible jellies can be produced also with barium hydroxide and magnesium hydroxide[108, 138] but the hydroxides of the other common heavy metals are all too nearly insoluble to serve as coagulants for the casein.

Formula **A** can be made water-resistant by adding calcium hy-

droxide[65, 148, 172] but in order to obtain a satisfactory working life it is advisable to increase the proportion of sodium hydroxide used. The following proportions are suitable:

G	Casein	100 grams	
	Water	250	
	Sodium hydroxide	11	(0.275 gram equivalent)
	Calcium hydroxide	20	(0.54 gram equivalent)

The glue can be mixed most quickly by soaking the casein in part of the water, and then adding the sodium hydroxide dissolved in the remainder of the water. The mixture should then be stirred thoroughly until the casein is completely dissolved. After adding the lime and stirring for a few minutes the glue is ready for use and remains workable for roughly 6 or 7 hours. It is capable of giving joints in woodworking of excellent dry strength and good water-resistance.

The maximum binding capacity of casein for alkalies, as pointed out in Chapter 3, is commonly considered to be about 180×10^{-5} gram equivalents per gram, which would be 6.7 grams of calcium hydroxide per 100 grams of casein. Formula **G** calls for three times that amount of calcium hydroxide because it is found necessary for adequate water resistance.[165] The opacity of water-resistant casein glues shows clearly that they contain a dispersed solid phase, *i.e.*, the excess calcium hydroxide and other calcium salts, which is held in dispersion by protective action of the casein and is essential for good water-resistance. Water-resistance, therefore, is not so much a matter of forming an "insoluble" calcium caseinate as it is one of peptizing a solid phase of very low solubility within the colloidal structure of the jelly. In this connection the theories of coagulation of milk with rennet and the firmness of the resulting curd as discussed in Chapter 1 should be reviewed.

In formula **G** the proportion of sodium hydroxide may be varied between 4 and 12 grams; but at the lower proportions of sodium hydroxide the proportion of water must be increased materially if the viscosity of the glue is to be kept constant, and the working life of the glue is much shorter.

When ammonium hydroxide is used as the alkali in place of sodium hydroxide it is found, as would be expected, that between 1½ and 2 times as much water must be added to obtain a glue of the same viscosity. Such glues are capable of giving wood joints of good dry strength and water-resistance and would therefore be very economical to use, but unfortunately the working life is inconveniently short unless the amount of lime is decreased so much that water-resistance is impaired. The reason for the short working life lies in the low pH of such glues in consequence of the weak basic properties of ammonium hydroxide.

For reasons that have already been discussed, formula **G** is not

satisfactory for making prepared casein glues. Formula **E** may be taken as the starting point for developing water-resistant prepared casein glues. By increasing the content of calcium hydroxide considerably above that necessary for reaction with the compound sodium X, the water-resistance is improved materially, but at the same time the working life is cut down to about two hours. To extend the working life it is necessary to make a more alkaline glue by increasing the proportions of both lime and sodium X. A suitable formula is:

	Casein	100 grams	
H	Calcium hydroxide	30	(0.81 gram equivalent)
	Sodium X	0.275 gram equivalent	

The three dry ingredients can be ground together, packed, shipped, and stored until the glue is needed for use. The powder is merely added to water in such proportions as to give about 300 grams of water per 100 grams of casein and stirred thoroughly until the casein dissolves. The glue has a working life of about 6 or 7 hours and is capable of giving strong joints with good water-resistance.

Among the sodium salts that may be used for sodium X in formula **H** are: sodium fluoride,[62, 78, 106, 114, 130, 133, 173, 175, 177, 178, 184, 185, 196, 198] disodium phosphate,[114, 139, 156] trisodium phosphate,[66, 67, 78, 114, 117, 156, 160, 171, 175, 176, 177, 178, 181, 183, 185, 198] sodium sulfite,[117, 174, 176, 181, 183, 184, 196, 198] sodium hyposulfite,[171] sodium carbonate,[27, 69, 75, 156] borax,[156, 162] sodium arsenate,[182] sodium molybdate,[183] sodium tungstate,[52] sodium aluminate,[179] sodium stannate,[41] sodium silicate,[46, 48, 52, 62, 106, 114, 123, 156, 162, 171, 179, 181, 185] sodium formate,[174, 184, 185] sodium oxalate,[139] sodium tannate,[178] and sodium citrate, tartrate, salicylate, and resinate.[41] In most of the patents cited a mixture of two or more of these salts is used for sodium X and in some of them there is also an addition of sodium hydroxide.[62, 75, 156, 172, 176, 179] Potassium or other alkali metal salts or ammonium salts can be used in place of sodium salts[156] but the viscosity of the resulting glue will differ as has already been pointed out.

The viscosity of glues of formula **H** can be altered through wide limits by varying the proportions of water used, a matter that must be considered very carefully with relation to the conditions under which the glue is to be used. The working life of the glue is somewhat longer, the greater the proportion of water. Another method of altering the viscosity without changing either the amount of water or the alkalinity is by adding certain salts such as alum,[101, 174] calcium chloride[171, 175, 177, 180, 183] sodium chloride,[172, 173] or sodium sulfate.[182] Small proportions of such salts, preferably added after the glue has been mixed, may increase the viscosity materially without affecting the working life to any great extent. In the presence of excess calcium hydroxide and

at the alkalinity prevailing in glues of formula **H**, calcium chloride does not precipitate the casein promptly as a curd, as it does in sodium caseinate solutions containing little or no calcium hydroxide.

In formula **H** the proportion of calcium hydroxide can be altered within very wide limits without affecting the working life seriously. It can vary from approximately 17 grams (0.46 gram equivalent) to at least 50 grams. As the proportion of calcium hydroxide is decreased below 17 grams the working life soon begins to increase rapidly but the water-resistance is impaired. Since 0.275 gram equivalent of calcium hydroxide is consumed in the reaction with sodium X, at the above limit only 0.185 gram equivalent is available for reaction with the casein. This is about the amount ordinarily considered necessary to combine with 100 grams of casein to form the "completely saturated" caseinate. The longer time necessary for gelation as the proportion of calcium hydroxide is decreased below this limit, therefore, may be accounted for by considering that only a fraction of the total casein present has been converted to the gel-forming calcium compound. That view is further substantiated by the fact that as the content of calcium hydroxide is decreased below that limit the glue usually loses the white, turbid appearance characteristic of calcium caseinate (in milk, for example) and takes on more of the clear amber-colored aspect of sodium caseinate solutions such as formula **A**. The independence of the working life and the content of calcium hydroxide above the limit given is to be expected because the limited solubility of calcium hydroxide keeps the proportion in solution constant regardless of that present in the solid phase.

The water-resistance of the glue begins to be impaired by cutting down the proportion of calcium hydroxide in formula **H** before the limit of 17 grams is reached; in fact, if a high degree of water-resistance is desired the content of calcium hydroxide should not be materially less than that given in the formula. On the other hand, this amount is sufficient for the purpose, larger proportions not increasing the water-resistance to any important degree.

Variation in the proportion of sodium X in formula **H** has much the same effect on the water requirement for constant viscosity and on the working life as variation in the proportion of sodium hydroxide in formula **G**. This is to be expected inasmuch as sodium X is used together with sufficient calcium hydroxide primarily as an indirect means of producing sodium hydroxide when the glue is mixed with water.

The casein glue of the Middle Ages and probably of the ancients seems to have consisted of casein, water, and lime only. It is, in fact, possible to prepare a glue that is capable of giving excellent dry strength and water-resistance as follows:

J	Casein	100 grams	
	Water	300	
	Calcium hydroxide	16	(0.43 gram equivalent)

The casein must be fairly finely ground and must be allowed to soak thoroughly before adding the lime in order that solution may take place as readily as possible. Once mixed, the glue must be used without delay because the working life is limited to 10 to 45 minutes, depending largely upon the character of the casein used. Although the working life is so short, formula **J** was used for a time during 1917 in at least one factory making plywood for aircraft manufacture. Dunham[68] claims that the working life of formula **J** can be extended sufficiently to make a practicable prepared glue by adding animal glue.

Casein glues made in accordance with formula **H**, using sodium silicate for sodium X, differ radically from all the others. A much longer working life is obtained in a glue of given alkalinity, or expressed in another way, much less sodium hydroxide need be introduced to attain a given working life. A second important difference lies in the manner in which variation in the content of calcium hydroxide affects the working life. With the casein-silicate glues there is a considerable range of lime content (above that necessary to react with the sodium silicate) within which the working life decreases as the proportion of calcium hydroxide increases. With the other glues of formula **H** this region of inverse proportionality between lime content and working life is very much shorter.

Casein-silicate glues may be prepared in accordance with the following formula:[46, 93]

K	Casein	100 grams	
	Water	250	
	Calcium hydroxide	20 to 30	(0.54 to 0.81 gram equivalent)
	Water	100	
	Sodium silicate	70	(containing 0.2 gram equivalent Na_2O)

It is important that the calcium hydroxide be added to the casein suspension before the sodium silicate.

The following data are illustrative of the variation in working life of formula **K** with the content of calcium hydroxide: 48 hours at 9 grams, 20 at 12, 16 at 15, 11 at 22, 4 at 27, 3 at 35, and 1¼ at 60.

The outstanding differences in the characteristics of formula **K** and formula **H** must be attributed to the presence of the colloidal silica introduced with the sodium silicate in formula **K**. The sodium silicate most often used for making casein glue has a silica-soda ratio of 3 to 3.25 and a density of approximately 40°Bé. A typical analysis might be 8.9 per cent Na_2O, 29.0 per cent SiO_2. We may consider this to

be roughly 17.6 per cent Na_2SiO_3 and 20.3 per cent colloidal SiO_2. If we consider that the Na_2SiO_3 reacts with $Ca(OH)_2$ to form $CaSiO_3$, formula **K** contains 18 grams of colloidal silica per 100 grams of casein, presumably in a very highly dispersed condition. It is not surprising that the presence of this silica retards the formation of the casein jelly and prolongs the working life of the glue. Even the addition of dry sodium silicate after glue of formula **H** has been mixed prolongs the working life.[66]

Casein-silicate glue is very satisfactory as a wet-mix glue but sodium silicate has not proved practicable as the principal source of alkali in prepared glues. Attempts have been made to incorporate it in prepared glues by carefully dehydrating the sodium silicate in mixture with water-soluble gums or a small proportion of casein[64] or by kneading it with the casein and drying the mixture,[52] but they have not been very successful. The difficulty is that the dry sodium silicate dissolves again very slowly unless it can be kept within a narrow range of low moisture content. During storage of the prepared glue the sodium silicate is likely to become either too dry or so moist that the glue cakes in the container. Dry sodium metasilicate is likewise too slow in dissolving and fails to provide the colloidal silica on which formula **K** depends. According to patent claims, however, sodium silicate may be used in prepared glues in which there is also sodium hydroxide[179] or salts such as sodium phosphate[181, 185] to dissolve the casein promptly.

Formula **K** has certain advantages over formula **H**. The glue is considerably less alkaline and still has about the same working life. One result is that slightly more water is used to give a glue of the required viscosity. This, taken in conjunction with the cheapness of lime and sodium silicate, makes it a comparatively inexpensive casein glue. The lower alkalinity also reduces the rate at which hydrolysis proceeds.

Addition of a small proportion of a soluble copper salt to formula **K** has been found effective in obtaining uniformly high values for water-resistance. The modification is especially advantageous where it is desired to use somewhat less calcium hydroxide to prolong the working life and yet retain high water-resistance[25, 48, 85, 123, 185] (see also[22, 51, 96, 200]).

L	Casein	100 grams
	Water	220
	Calcium hydroxide	20 to 30
	Water	100
	Sodium silicate	70
	Copper chloride	2 to 3
	Water	30

After the casein has been brought into solution by the calcium hydroxide and sodium silicate, the copper chloride solution is added slowly and with constant stirring. As the solution mixes with the glue, the green copper hydroxide can be seen to precipitate, but in a few minutes the violet color characteristic of colloidal dispersions of copper hydroxide appears. The glue is then ready for use. Another method of mixing the glue is to add the copper chloride to the water in which the casein is soaked before adding the suspension of lime. The presence of the copper compounds seems to have little direct effect on the working life of the glue; if anything, it extends it slightly. By permitting the use of less lime, it extends the life indirectly.

Miscellaneous Ingredients of Casein Glue

The addition of carbon disulfide to casein glues after they have been mixed increases the water-resistance.[1, 53, 150] Wood joints made with such glues are about 10 per cent stronger when tested wet than joints made with the same glue without carbon disulfide. Other sulfur compounds such as thiocarbanilide, thiocarbonates, and xanthates[1] and sulfur[100] are said to have the same effect. Barium peroxide[99] is also said to improve water-resistance.

Non-drying oils are sometimes added to prepared casein glues to prevent dusting of the dry powder during handling and mixing and to retard change of calcium hydroxide to calcium carbonate by contact with air. Mineral oil such as kerosene[114, 148, 160, 171, 179, 182, 183, 184, 185] or turpentine,[12, 133, 175] pine oil,[114] or castor oil[171,178,185] is used for the purpose. Oil of citronella[175] is proposed as a perfume. Addition of linseed oil,[212] cashew nut shell oil,[120] rosin,[160, 173, 182] and portland cement[130] have been patented. Camphor,[52, 211] dextrine,[172, 173, 180] starch,[23, 26, 172,177] powdered ivory nut,[78] and lecithin[102] are proposed, for purposes best known to the inventors. Magnesia,[172, 177, 181, 184] magnesium carbonate,[198] and aromatic sulfonic acids[25] are said to prolong the working life, and zinc oxide[184] is said to delay hydrolysis. Glycerol is sometimes added to make the dry glue more flexible.[219] Amides such as urea, in proportions of 15 to 100 parts per 100 parts of casein,[79, 203] are proposed as solvents for casein in casein glues, and butylated naphthalene sulfonic acid and similar compounds containing not less than 12 carbon atoms[220] and emulsifying agents of the alkyl, cycloalkyl, and aryl groups[104] are said to hasten solution in alkalies. A very concentrated aqueous solution of ammonium thiocyanate is also patented as a solvent for casein in casein glue.[169]

Glues can be made in which casein is mixed with other organic colloids. For example, mixtures of casein have been made with the following: animal glue,[68, 201] blood albumin,[77, 114, 141] seed meals such as soybean, peanut, and cottonseed meal,[26, 27, 77] rubber latex,[20, 79, 159, 223]

and synthetic resins.[18, 50] The mixtures with blood albumin and with seed meals have found some commercial use in woodworking and those with latex and synthetic resins in other industries.

Control of Consistency and Working Life

Formula G

Figure 30 shows the changes in viscosity during the working life of five glues of formula **G** but with varying alkalinity ranging from 4 to

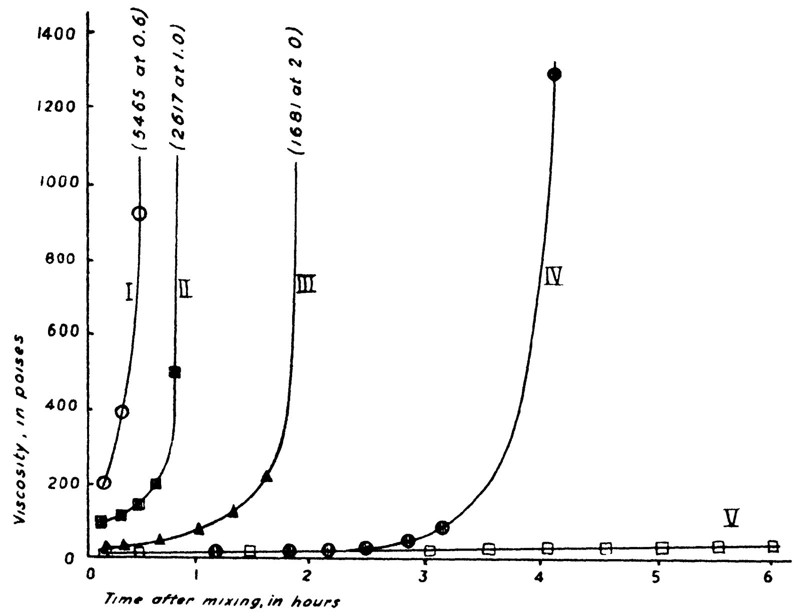


Figure 30.—Change in viscosity with time after mixing for casein glues of type formula **G** but with varying alkalinity. The glues all contained 100 grams of casein, 300 grams of water, 15 grams of calcium hydroxide, and sodium hydroxide as follows: 4 grams in I, 6 grams in II, 8 grams in III, 10 grams in IV, and 12 grams in V.

12 grams (0.1 to 0.3 gram equivalent) of sodium hydroxide per 100 grams of casein. Such glues are truly viscous and their viscosity is adequately expressible in poises, whereas glues of formula **F** are usually pseudoplastic.[39] Ten minutes after mixing, the viscosities of the glues with 4, 6, 8, 10 and 12 grams of sodium hydroxide are respectively 200, 90, 20, 6, and 5 poises. Since the desirable viscosity for most woodworking purposes is 20 to 100 poises, the least alkaline glue should be mixed with more water and the two most alkaline glues with less water. The pH of the five glues, measured by the hydrogen electrode,

is 12.19, 12.44, 12.68, 13.08, and 13.25 respectively,[41] which represents the practical limits of pH range of casein glues of formulas **G** and **H**. It is clear that this range lies on the downward slope to the right of the alkaline maximum of the viscosity-pH curve of Figure 5 and that beyond pH 13 there is little further change in viscosity with pH.

The viscosity of casein glues containing calcium hydroxide changes with time after mixing. There may be a decrease in viscosity during the early part of the working life; but if so it reaches a minimum after which it increases, at first slowly, then ever more rapidly until, if the glue is not too strongly alkaline or too dilute, the viscosity shoots up rapidly to enormous values and the glue forms a jelly. The time elapsing between mixing the glue with water and the rapid increase in viscosity and formation of a jelly is the working life of the glue. Very strongly alkaline glues like glue V of Figure 30 do not form jellies unless mixed with much less water. When no jelly forms the viscosity passes through a minimum, then through a maximum and finally the viscosity falls off to very low values. (On the scale of ordinates used for Figure 30 the minimum in the early part of the working life is not observable and the abscissas do not continue to the

TABLE 21.—Strength and Water-resistance of Plywood Glued with a Moderately Alkaline and a Very Alkaline Casein Glue at Different Ages during the Working Life

Age of glue when joints were made (hrs.)	Moderately alkaline glue — Strength of joints (lbs./sq. in.) when Dry	Wet	Very alkaline glue — Strength of joints (lbs./sq. in.) when Dry	Wet
1	440	100	413	186
2	459	176	354	182
3			390	149
4			380	123
5	456	179	326	0
7			235	0
23			28	0

time when the maximum occurs.) Although very alkaline glues seem to have a long working life, only the early part of their working life is safely usable because the strength and water-resistance of joints made with them decrease rapidly during the life of the glue as shown in Table 21.[41] Only those casein glues that have a limited working life and form firm jellies are reliable, especially where water-resistance is required.

The changes in consistency of casein glue can be followed after the jelly is formed by means of an instrument for measuring jelly strength. The Bloom gelometer, commonly used for animal glue,[55] is suitable. At 27°C. the jelly strength usually continues to rise rapidly to a maximum at an age of 8 to 12 hours after the glue was mixed. After pass-

ing the maximum, the jelly strength generally declines slowly until the glue reliquefies within a few days, provided that the jelly is not allowed to lose its moisture by evaporation in the meantime. The curves of viscosity vs. time and jelly strength vs. time taken together are highly characteristic of different formulations and are called the consistency history of the glue. Specifications for casein glue probably could be written largely in terms of consistency history just as specifications for animal glue are written in terms of viscosity and jelly strength.[81]

Glues of formula **G** but with varying alkalinity were mixed with enough water to give a minimum viscosity of 50 poises during the working life, and the consistency history observed.[41] With 6, 8, 9, 10, and 12 grams of sodium hydroxide the amount of water used was 340, 300, 280, 260, and 260 grams respectively and the maximum jelly strength was respectively 210 grams after 8 hours, 200 grams after 9 hours, 150 grams after 10 hours, 85 grams after 11 hours, and 35 grams after 12 hours. The higher the alkalinity the longer is the working life, the longer it takes to reach the maximum jelly strength, the weaker the jelly strength at its maximum, and the sooner the jelly reliquefies. When a glue of any given degree of alkalinity is mixed with varying proportions of water, the minimum viscosity is lower, the working life longer, and the maximum jelly strength lower, the greater the proportion of water; but the maximum in jelly strength is attained at the same age.

The consistency history of the water-resistant casein glues is chiefly the resultant of two opposing processes, gelation of calcium caseinate on one hand and alkaline hydrolysis of casein on the other. The increase in viscosity toward the end of the working life and the rise to maximum jelly strength are governed by gelation, which is opposed by hydrolysis inasmuch as the hydrolytic products do not form jellies. The maximum of jelly strength marks the point at which the velocity of hydrolysis begins to dominate over that of gelation. Gelation apparently proceeds more rapidly the higher the concentration of undecomposed casein, and the higher the concentration of calcium ions in solution. The concentration of calcium ions is determined by the solubility of calcium hydroxide, an excess of which is always present. The solubility of calcium hydroxide is lower, the higher the alkalinity because of the effect of mounting hydroxyl ion concentration on the solubility equilibrium. Gelation is therefore slower at higher alkalinities and in the meantime hydrolysis, which is accelerated at higher alkalinities, more seriously reduces the concentration of undecomposed casein.

The attainment of reasonable working life together with satisfactory joint strength and water-resistance depends upon a nice balance between these two tendencies. The joint must be made and dried to a reasonable extent (drying at first proceeds largely by absorption of the glue water

by the dry wood) before hydrolysis proceeds too far, say much beyond the age at which the maximum jelly strength is attained.

Since dry joints made with casein glue retain their water-resistance for years, it is clear that hydrolysis does not occur in reasonably dry jellies of casein glue. If the glue jelly subsequently becomes wet, however, hydrolysis again sets in. Thin films (about 0.005 inch thick) of casein glues of formula **G** with varying alkalinity were prepared by spreading the glues on ferrotype plates previously lubricated with Halowax (chlorinated naphthalene), letting them dry for 24 hours in a room at 90 per cent relative humidity, then stripping the films from the ferrotype plates, cutting them into suitable test specimens, and finally letting them dry in a room at 30 per cent relative humidity. The dry films when tested for strength in tension in a Schopper paper testing machine broke at loads of 2,200 to 2,300 pounds per square inch independently of the alkalinity, and retained that strength indefinitely. When dry specimens were placed in a room at 95 per cent relative humidity, where they reached equilibrium moisture content in a few hours (see Figure 29), and were tested in the Schopper machine at intervals, the results given in Table 22 were obtained. The films weaken faster, the higher the alkalinity of the glue. In another test the films were hung in 95 per cent relative humidity under a dead load of 10 pounds per square inch and the elongation measured at intervals until the films broke. The films containing 4, 6, 8, 10, and 12 grams of sodium hydroxide per 100 grams of casein showed respectively 20 per cent elongation after 141 hours, 25 per cent after 87 hours, 29.4 per cent after 72 hours, 37.5 per cent after 48 hours, and 37.4 per cent after 48 hours and broke shortly thereafter.[41]

TABLE 22.—Rate of Loss in Strength of Thin Films of Casein Glue in 95 per cent Relative Humidity[41]

Time exposed (days)	Average strength in tension of films of glue of formula G containing the indicated proportions of sodium hydroxide (lbs. per sq. in.)			
	6 grams	8 grams	10 grams	12 grams
0	2,238	2,171	2,316	2,292
3	2,110	1,956	1,652	1,477
6	2,110	1,938	1,167	541
12	845	1,063	616	294
18	98	films too weak to test		

Formulas H and K

The general trends discussed for formula **G** apply likewise to formula **H** except that the insoluble CaX_2, formed by reaction between sodium X and calcium hydroxide, exerts a measurable effect on the consistency history of the glue, the nature of which effect depends upon the anion X. Although the CaX_2 is present almost entirely as a solid phase, it is

precipitated in the presence of dissolving casein. It is held in colloidal dispersion by the protective action of the casein and undoubtedly takes part in the subsequent formation of the jelly. Not until the jelly reliquefies and the casein reaches an advanced stage of hydrolysis does the CaX_2 settle out.

Figure 31 shows the relation between the working life and the alkalinity of casein glues of formulas **G**, **H**, and **K**.[41] If the CaX_2 had no effect

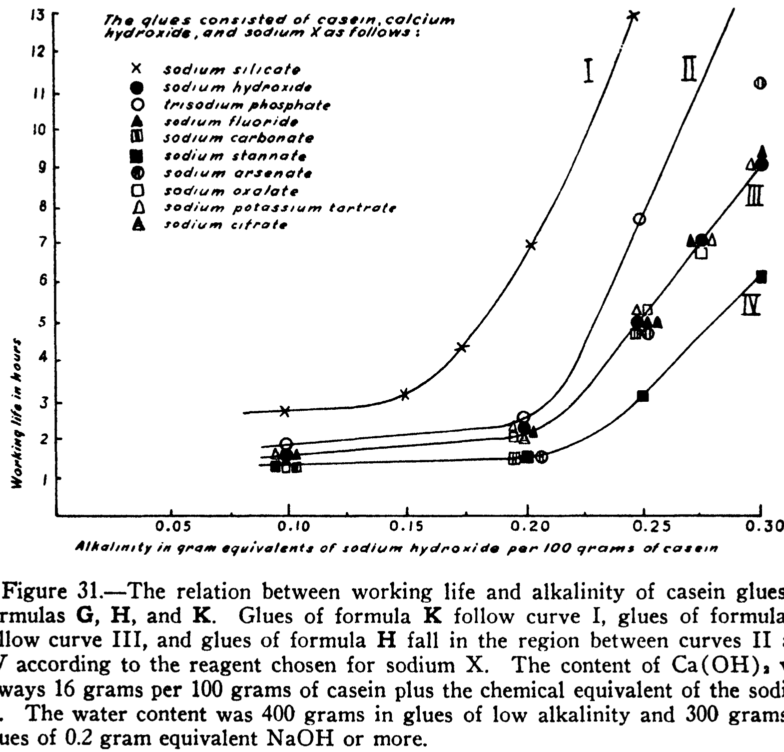


Figure 31.—The relation between working life and alkalinity of casein glues of formulas **G**, **H**, and **K**. Glues of formula **K** follow curve I, glues of formula **G** follow curve III, and glues of formula **H** fall in the region between curves II and IV according to the reagent chosen for sodium X. The content of $Ca(OH)_2$ was always 16 grams per 100 grams of casein plus the chemical equivalent of the sodium X. The water content was 400 grams in glues of low alkalinity and 300 grams in glues of 0.2 gram equivalent NaOH or more.

on the properties of the glue, the relation should be the same for all these glues, namely, Curve III. Most glues of formula **H** do follow Curve III fairly closely but others depart from it rather seriously. Those made with trisodium phosphate follow Curve II, exhibiting longer working life than would be expected from the stoichiometric alkalinity, and at the highest alkalinities failing to form jellies at all. Glues made with sodium stannate follow Curve IV, having shorter working life than would be expected, while glues made with sodium arsenate have shorter working life at low alkalinities and longer working life at high alkalinities. By using mixtures of reagents for sodium X, any part of the

region between Curves II and IV can be realized with formula **H**. Formula **K**, following Curve I, exhibits markedly longer working life at all alkalinities. The sodium silicate used in the experiments was one with a ratio of 3.25 equivalents of SiO_2 per equivalent of Na_2O; if sodium silicate of lower ratio of SiO_2 to Na_2O is used the curve falls below Curve I, but even with sodium metasilicate it still lies well above Curve III.

The different reagents chosen for sodium X likewise exert specific effects on viscosity and jelly strength but the effects on viscosity and on jelly strength are not necessarily parallel. Sodium fluoride and sodium tartrate, for example, lower the viscosity; but the former makes weaker jellies and the latter stronger jellies than glues of the same alkalinity made with sodium hydroxide. Sodium citrate leaves the viscosity practically unchanged but increases the jelly strength, while sodium oxalate changes neither viscosity nor jelly strength; but at very high alkalinities it fails to form a jelly at all. Sodium resinate and sodium oleate make glues that are strongly pseudoplastic rather than viscous and yield very strong jellies. Trisodium phosphate makes glues with very high viscosity in the early part of the working life, so that they are hard to mix properly, but after an hour or so the viscosity becomes moderate and the working life is relatively long, the jellies weak, and at very high alkalinity no jelly is formed. Sodium silicate increases viscosity and makes very strong jellies that remain strong for an exceedingly long time.

That these effects are due largely to the insoluble CaX_2 formed in the glue can be shown by mixing glues according to formula **G** and then adding solid substances.[41] Thus when calcium fluoride and calcium phosphate are added in the proportions in which they would be formed, the variations in consistency history observed in the fluoride and phosphate glues of formula **H** are reproduced, with the exception of the high initial viscosity of the phosphate glue. Nearly all solids, when added in sufficient quantity, increase both viscosity and jelly strength. Rosin makes exceedingly strong jellies. Such substances as lampblack, bentonite clay, and wood flour make strong jellies, and white lead makes strong jellies that remain reasonably strong for many weeks. Silica, however, failed to raise the jelly strength or to exert any of the effects characteristic of glues of formula **K**, made with sodium silicate. Dry, ground silica and the colloidal silica formed from sodium silicate are essentially different in behavior. There seems to be much similarity between the action of solids on casein jellies and the action of pigments in linseed-oil paints.

The reason for the sharp bend in Curves II, III, and IV of Figure 31

at a stoichiometric alkalinity of 0.2 gram equivalents of sodium hydroxide per 100 grams of casein is revealed in a study of the pH of casein glues as measured with the hydrogen electrode, Table 23. Below alkalinity 0.2 most of the sodium hydroxide is combined with the casein and the pH is approximately that of the saturated solution of calcium hydroxide. Above alkalinity 0.2 the pH begins to increase much more rapidly, very

TABLE 23.—The pH of Casein Glues of Formulas **G**, **H**, and **K** with Varying Proportions of Available Sodium Hydroxide[41]

Casein 100 grams; *Calcium hydroxide* 15 grams plus the chemical equivalent of the sodium X; *Sodium X* enough to provide the amount of available sodium hydroxide (alkalinity) indicated in the column headings; *Water* 400 grams for glues of alkalinity up to 0.2 and 300 grams for higher alkalinities

	pH at the alkalinity indicated				
	4	6	8	10	12 grams
Sodium X	0.10	0.15	0.20	0.25	0.30 gram equiv.
Sodium hydroxide ...	12.31	12.50	12.71	13.08	13.25
Sodium fluoride......	12.32	12.48	12.74	13.13	13.23
Sodium citrate......	12.41		12.69		12.91
Sodium stannate.....	12.30		12.70		13.09
Trisodium phosphate.	12.40	12.67	13.08	13.24	13.30
Sodium silicate......	11.95	11.99	11.95	11.95	11.95

little more sodium hydroxide combines with the casein, and most of the additional sodium hydroxide remains free in solution. If the proportion of NaOH combined with the casein is calculated from the pH by the method of Hoffman and Gortner[128] and plotted against the alkalinity, a curve breaking sharply at alkalinity 0.2 is obtained.[41] Below alkalinity 0.2 dilution of the glue with water increases the pH slightly, whereas above 0.2 it decreases it slightly. The critical alkalinity of 0.2 is a little more than the generally accepted value for the maximum binding capacity of casein, namely 0.18 gram equivalents per 100 grams. Apparently the rate of gelation is nearly independent of the alkalinity until the capacity of the casein for binding sodium hydroxide is exceeded. When the solution contains much free sodium hydroxide, however, gelation is retarded, presumably because the concentration of calcium ion is repressed while the rate of hydrolysis is accelerated.

The data of Table 23 show that at low alkalinities the pH is practically independent of the particular salt chosen for sodium X, except in the case of sodium silicate, but that at high alkalinities there are small but significant variations in pH sufficient to account at least in part for the spread between the Curves II, III, and IV in Figure 30.

As a rule the pH of casein glues is reasonably constant throughout most of the working life, but when they are first mixed the pH is relatively low and increases during the first hour. The characteristic decrease in viscosity to a minimum during this time undoubtedly arises

from this change in pH. Glues made with trisodium phosphate, which are exceedingly viscous when first mixed and exhibit an unusually deep minimum before the viscosity begins to rise, are exceedingly low in initial pH. Table 24 shows the changes in pH during the working life of three glues of equal alkalinity, 0 25 gram equivalent, but made respectively with sodium hydroxide, sodium fluoride, and trisodium phosphate. The first two glues mix with water easily and promptly, but the last is difficult to mix because it passes through a stage of unmanageable consistency during the first half hour. It seems probable that the trisodium phosphate at first forms a calcium sodium phosphate, releasing only part of the available sodium hydroxide, and yields tricalcium phosphate and the remainder of the sodium hydroxide only after some time has elapsed. Toward the end of the working life, on the other hand, the phosphate glue is relatively high in pH, corresponding to its long working life and relatively low maximum jelly strength.

Glues of formula **K**, made with sodium silicate, differ fundamentally from glues of formulas **G** and **H**, as revealed by the following facts: Curve I of Figure 31 bends at 0 15 instead of 0 20 gram equivalent of available sodium hydroxide; the pH as shown in Table 23 is lower than that of other glues and does not change with increasing alkalinity; nearly all the sodium hydroxide remains bound even when there is as much as

TABLE 24 —Change in pH During the Working Life of Three Casein Glues of Equal Alkalinity but Made with Different Reagents[41]

Casein 100 grams; *Calcium hydroxide* 15 grams plus the equivalent of the sodium X *Sodium* X enough to produce an alkalinity of 0 25 gram equivalent, *Water* 300 grams

Age of glue hours	pH of glue made with		
	NaOH	NaF	Na_3PO_4
0 25	13 02	13 04	
0 33	13 05	13 08	
0 50	13 06	13 10	11 34
0 75	13 06	13 12	12 56
1 00	13 07	13 13	12 96
1 25	13 07	13 13	13 10
1 50	13 07	13 13	13 18
1 75	13 07	13 13	13 24
2 00	13 07	13 13	13 28
3 00	13 09	13 13	13 36
4 00	13 12	13 13	13 36
5 00	(set to jelly)	13 13	13 36
6 00		(set to jelly)	13 36

0.6 gram equivalent per 100 grams of casein; the working life is inversely proportional to the content of calcium hydroxide even at high contents of lime; the maximum jelly strength is high and remains high for a very long time; and the jellies have some of the inelastic qualities of silica

gel. The two colloids of acid reaction, silicic acid and casein, both take part in the combination with alkalies and in the region of concentration, pH, and content of calcium hydroxide in question, form interlocking and irreversible jellies.

Effect of Grade of Raw Materials on Properties of Glue

Casein

Glue can be made successfully with commercial caseins of any type, from grain curd or continuous process to rennet, and with casein from skim milk or from buttermilk; but the different caseins cannot be used indiscriminately because slight but important changes in the glue formula must be made when changing from one kind of casein to another. The glue maker's principal requirement is uniformity in the properties of the casein from shipment to shipment. Casein of medium to low ash content is usually preferred.

The methods of proximate analysis described in Chapter 5 afford a reasonably satisfactory basis for testing casein for uniformity and quality and for drawing up specifications for purchase.[2, 8, 36, 54, 112, 154, 192, 202] They should, however, be supplemented by preparation and testing of a laboratory batch of glue according to the formula in which the casein is to be used. The glue may be tested by observing the consistency history, including both viscosity and jelly strength, and by making tests of dry and wet joint strength.

The proximate ash content of a casein is a fairly reliable index of the relative viscosity of glue made with it according to any given formula, or, from the more practical point of view, of the proportion of water required to make glue of a given viscosity.[47, 163, 199] Within reasonable limits, the relation between water required for given viscosity in any given formula and proximate ash content of the casein is linear. For formula **G** it may be expressed by the equation $W = 1.65 + 0.20A$, for formula **H** by $W = 2.45 + 0.13A$, and for formula **K** by $W = 1.85 + 0.24A$, in which W is the ratio of grams of water per gram of casein and A is the proximate ash content in per cent. It is important to note that, for this relation to hold, the ash content of caseins low in ash must be determined by simple incineration without addition of base to fix the organic phosphorus.

According to Dunham,[76] caseins high in ash content, including rennet casein, can be made to yield solutions of low viscosity by soaking the casein, after it has been precipitated, in aqueous phosphoric acid, hydrofluoric acid, or hydrofluosilicic acid, and then drying it. Presumably the ash content is reduced by the treatment.

The method by which the casein is manufactured seems to be relatively

unimportant except as it affects the ash content because the relation between water requirement and ash content holds good from pure casein of very low ash content, through the various commercial caseins, to rennet casein of very high ash content. In solutions of casein containing no lime and in a lower range of pH, such as glues of formula **F**, the linear relation between water requirement and ash content probably does not hold. For such glues Zoller[229] finds that the viscosity is higher, the higher the temperature to which the curd has been heated during the manufacture of the casein. The borax solubility test is an index of the behavior of a casein in formula **F** but not of its behavior in formulas **G, H, K,** or **L.**

The user of a wet-mix casein glue can alter the proportion of water in accordance with the ash content or the viscosity of different lots of casein. The manufacturer of a prepared glue, however, finds it impracticable to change his directions for mixing and therefore must either see that his supply of casein runs uniform in ash content or alter his formula in some way to keep the viscosity constant when the powder is mixed with water in the prescribed proportions. He may, within limits, vary the proportion of calcium hydroxide or of some inert ingredient to change the ratio of water to casein although that of water to dry powder remains constant; or he may change the proportion of sodium X to alter the pH and thereby control the viscosity.

The working life of the glue tends to be shorter the higher the ash content of the casein even when the proportion of water is adjusted to hold the viscosity constant. The relation between working life and ash content, however, is not so reliable as that between viscosity and ash content, as is illustrated by Table 25.[47]

TABLE 25.—The Relation between Working Life of Glue and Ash Content of Casein, for Glues of Formula **K** and Approximately the Same Initial Viscosity

Kind of casein	Proximate ash content (%)	Ratio of water to casein	Working life (hrs.)
Grain curd	1.28	2.2	12
Lactic acid	1.43	2.2	11
Lactic acid	1.70	2.3	16
Lactic acid	2.51	2.4	10
Lactic acid	2.62	2.5	6½
Sulfuric acid	2.64	2.5	14
Grain curd	2.70	2.5	9
Sulfuric acid	4.29	2.9	6½
Sulfuric acid	5.06	3.0	6
Sulfuric acid	6.03	3.3	4½
Rennet	8.59	3.9	5

High content of fat in casein has long been considered objectionable for glue making. This impression was probably carried over from previous experience in using casein for coating paper, where fat leads to difficulties.

It has already been pointed out that fatty oils are often added to casein glues for one reason or another. Recent investigations in Germany, where substitution of buttermilk for skim milk casein apparently is desired, indicate that high content of fat in the casein is not disadvantageous in making glue.[195] About one-fifth of the butter fat in casein is free fatty acid, which promptly forms soaps with the alkali of the glue and brings about emulsification of the neutral fat in very fine droplets.

Grade of Lime

The grade of the lime used in making casein glue seems to be without important effect on the properties of the product provided that the proportion of lime taken is calculated on the basis of its content of calcium hydroxide. The grade known in the trade as chemical lime is preferred.

It is most convenient to use slaked lime for casein glue because it is already finely powdered and also because the use of quicklime would increase the amount of heat evolved on mixing the glue. Most casein glues evolve enough heat during mixing to become sensibly warmer than the temperature of the ingredients. The heat results from chemical reaction taking place in the mixture on adding it to water, from the heat of swelling of the casein, and from mechanical work performed by the stirring. Since the viscosity of casein glue decreases rapidly as the temperature is raised and the working life also decreases, it is undesirable to contribute further to the heating of the glue by using quicklime.

The lime should be well calcined and carefully slaked. If it is not, small particles often remain suspended in the glue, take no part in the reactions, and are found later in the glue joint as undissolved particles.

The small percentages of carbonate in commercial slaked lime can be neglected in calculating the proportion to use in a given formula.

Grade of Sodium Silicate

The sodium silicate commonly used for making wet-mix casein glues has a ratio of Na_2O to SiO_2 of approximately 1 to 3.25 and a specific gravity of 41°Bé. Commercial sodium silicate varies widely in composition, from a grade having a ratio of soda to silica of 1 to 3.86 and a content of total solids of 31.1 per cent to a grade with a ratio of 1 to 1.58 and 62.5 per cent total solids. The products, however, are adequately identified by grade so that the desired kind can be purchased without difficulty. As already pointed out, the grade of sodium silicate makes an important difference in the properties of the glue.

Technic of Using Casein Glue for Woodworking

Mixing Casein Glue

Directions for mixing prepared casein glues in preparation for application are furnished by the manufacturer and should of course be

followed very closely. It is especially important to measure out the proper quantity of the powder by weight rather than by volume because the consistency of the glue may be altered to a serious extent if the proper ratio of water to glue is not obtained. In general the directions call for placing the water in the bowl of a suitable power mixer, then adding the powder slowly with constant stirring. Usually the stirring is continued until the glue has dissolved to a homogeneous mixture in which close inspection reveals no undissolved particles of casein. With some glues, however, it is advised to add the powder to the water and stir only long enough to distribute the glue through the mass of water uniformly and then to let it stand quietly for some minutes to permit the chemicals to dissolve and react. The mixture is then agitated to complete solution of the casein. A glue that passes through an exceedingly thick stage in the course of solution can often be handled to best advantage in this way.

Wet-mix glues of formulas **K** and **L** are mixed as follows:[93] "The casein and water are weighed into the bowl of the mixing machine and stirred sufficiently to distribute the water throughout the casein. If the casein is ground to pass a 20-mesh sieve it should be allowed to soak for 15 to 30 minutes before proceeding with the preparation. If the casein is more finely ground the soaking period may be reduced. In a separate container the hydrated lime is mixed with water. This mixture is stirred vigorously at first, but just before it is added to the casein it should be stirred with a gentle rotary motion, just enough to keep the lime in suspension. With the mixer in operation, pour the milk of lime into the casein-water mixture.

"Casein and lime act peculiarly. At first they form large, slimy lumps, which are balls of dry casein coated with partly dissolved casein. These break up rapidly, becoming smaller and smaller, and finally disappear. The solution, in the meantime, becomes somewhat thinner. A stirring device should be provided that insures thorough agitation. If a deposit of casein remains unacted on it may cause lumps later.

"When about one minute has elapsed since the lime and casein were united, it will be noticed that the glue has begun to thicken a little. The sodium silicate must be added now, or the glue will be too thick. Disregarding lumps, if they are but few, pour in the sodium silicate. The glue will thicken momentarily, but the stirring should be continued until the glue is free from lumps. This should not take more than 15 or 20 minutes, counting from the time the lime was added. If the glue is a little too thick, a small amount of water may now be added. If the glue is too thin, it will be necessary to start over again, using a smaller

proportion of water; hence it is important to make certain that too much water is not added originally.

"The copper salt may be added at any one of several times during the mixing process. A convenient method, recommended for general practice, is to dissolve the salt and stir the solution into the moistened casein immediately before the lime is added. The solution of the copper salt may be added at the end of the mixing process, but some care will be

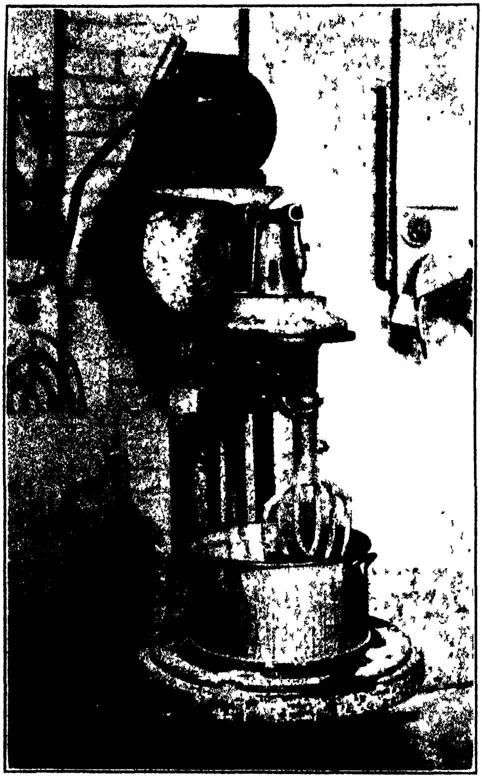


Figure 32 —
A casein glue mixer

Courtesy of the Forest Products Laboratory

required to avoid lumping. If so added the solution of copper salt should be poured slowly into the glue with continuous stirring. The mixing should be continued until any lumps that may have formed are broken up and a smooth, evenly colored glue is obtained. The dry copper salt may be added to and mixed with the dry casein, but if the casein containing the copper salt is soaked for an extended period a chemical action will take place between the copper salt and the metal container."

The consistency and working life of casein-silicate glues depend not

only upon the amount of water used, but also upon the time interval between the addition of the lime and of the sodium silicate; the longer the time elapsed, the thicker the glue and the shorter the working life. The working life is also shortened by having the glue room too warm.

Proper mixing of casein glue is essential for successful use. Even for laboratory batches a power mixer is desirable and for efficient mixing of batches it is necessary. The mixers commonly used for animal and vegetable glues are not always so suitable for casein glues. The type of machine used for mixing such materials as bakers' dough will generally be found satisfactory. The paddle should pass as close as practicable to the bottom and edges of the bowl to scrape off any glue tending to stick to the surfaces. It is very convenient to have several speeds for the paddle and it has been found that a motion of rotation in opposite directions in two non-concentric circles gives excellent results. The bowl and paddle should be detachable for ready cleaning. The parts in contact with the glue should be made of a metal or other material not corroded by alkalies or by ammonia. Brass, copper, and aluminum are to be avoided. Figure 32 shows a type of mixer that has given satisfactory service.

With a properly designed mixer there should be little trouble with development of foam in the glue. Where such trouble is experienced, addition of anti-foaming agents to the water before adding the glue has been proposed. Carbon tetrachloride or kerosene containing 5 per cent of cresylic acid have been patented for the purpose.[24] Ordinarily no such precautions against foaming should be necessary.

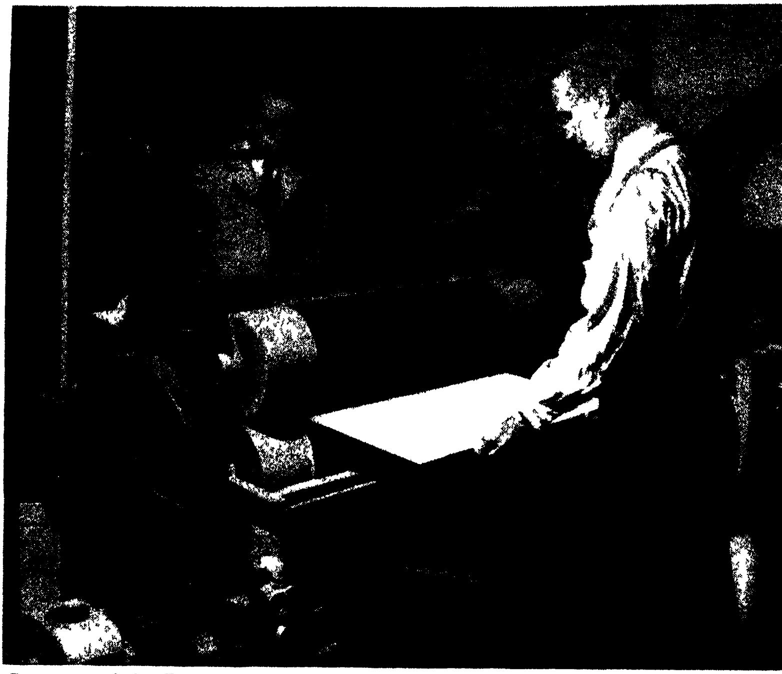
Applying Casein Glue

Casein glue can be spread by brush, but the brushes must be cleaned before the glue sets in the bristles because the glue jelly is very difficult to remove. The alkalinity is such that bristle brushes soon wear out. Fiber brushes wear longer. For these reasons some prefer to use wood or metal scrapers for applying the glue by hand. Its consistency is such that a tool of that kind can be employed very easily.

For large-scale production the mechanical glue spreaders used for other types of glue, such as the roll spreader shown in Figure 33, are entirely satisfactory for casein glue.

From the point of view of technic of application casein glue takes an intermediate position between vegetable and animal glue, resembling the former more closely than the latter. Like vegetable glue it is generally used cold on cold wood and does not change in viscosity as quickly as animal glue, though somewhat more rapidly than vegetable glue. As

usually prepared, casein glue flows somewhat more freely than vegetable glue.



Courtesy of the Forest Products Laboratory

Figure 33.—A mechanical spreader applying casein glue

Relation of Pressure Used to Gluing Conditions

In using any glue for woodworking the maximum joint strength is attained only if a proper correlation of pressure applied to the joint and consistency of the glue *at the moment of applying the pressure* is maintained. In general the more readily the glue flows the lower must be the pressure applied, and conversely, thick, jelly-like glue layers require a high pressure to develop strong joints. That statement is based upon a very large number of empirical data obtained under carefully controlled and widely varying conditions with casein, vegetable, and animal glues, and checked by observations of the gluing practices in woodworking factories extending over a number of years.[94, p. 169-183; 213, 215] If the gluing conditions are such that the film of glue in the joint at the moment of applying pressure is still very fluid and a high pressure is employed, it is found that a weak joint is obtained, because too much of the glue is either squeezed out or forced too deeply into the wood. Weak joints

of this kind are called starved joints.[86] An examination of a section of a starved joint under a microscope generally shows an absence of an appreciable glue film between the joined surfaces.[107] On the other hand, if a film of a hot glue such as animal glue has gelled before pressure is applied to the joint, a weak joint known as a chilled joint is obtained unless a comparatively high pressure is applied. A third type of weak joint known as a dried joint is obtained if the film of glue loses too much moisture by evaporation or absorption by the wood before pressure is applied.[87] Casein glues are subject to starved joints on one hand or dried joints on the other if the gluing technic is not correctly guided.

The consistency of the glue at the time of pressing depends not only upon its viscosity at the moment of applying it to the wood, but upon the changes in its condition taking place during the time necessary to assemble the pieces and apply the pressure. With animal glue, changes in consistency may take place very rapidly while assembling, in consequence of its property of gelling as it cools. The pressure must therefore be adjusted in accordance with the other gluing conditions with much precision. The changes taking place in casein glue during this time are less rapid because the temperature effect is eliminated. A given pressure may therefore remain a suitable one throughout a longer assembly period than would be the case with an animal glue of high grade.

Such latitude in gluing conditions is often very important to the woodworker. In making plywood, for example, the nature of the manufacturing operations makes it necessary to build up a stack containing a large number of panels before applying the pressure. There must consequently be a great difference in the assembly time for the first and the last panels in the stack, yet each receives the same pressure.

The factors, or gluing conditions, upon which the consistency of a casein glue layer in a wood joint at the time of pressing depends, include:

The consistency of the glue used
The spreading rate (area covered by a given amount of glue)
The kind of wood, its temperature, and its moisture content
The temperature of the room
The assembly time
Whether "open assembly" or "closed assembly" is used.

Obviously if other conditions are the same the consistency at the time of pressing is thicker, the more viscous the glue to begin with. Again, if a low spreading rate giving a deep layer of glue is used, evaporation and absorption of moisture by the wood produce less change in a given time than is the case with a shallow layer of glue. If the wood used is of a kind that absorbs water very rapidly, if it has a low moisture content, and if its ability to take up water quickly is favored by raising its temperature, there is a greater thickening of the glue layer during assem-

bly than under the opposite conditions. Loss of moisture and consequent change in viscosity of the glue layer are increased by raising the temperature of the room, prolonging the assembly time, and using open assembly. By "open assembly" as distinguished from "closed assembly" is meant a condition in which the glue layer after application is freely exposed to the atmosphere for a considerable portion of the assembly period instead of being covered promptly by the second of the two surfaces to be joined.

In the case of casein glue, as of vegetable and animal glues, it is common practice to apply the glue to only one of the two surfaces to be joined. Single spreading has been found to give just as satisfactory results as double spreading provided that a sufficient quantity of glue has been used per unit area of joint and that the other gluing conditions are properly adjusted.

The conditions under which casein glues are used are so diverse that it is difficult to give general recommendations about the combination of gluing conditions to be followed. Each user should work them out for his own operations by making careful tests. For reliable results under a wide range of gluing conditions the amount of glue spread in single spreading usually should be approximately 75 pounds of wet glue per 1,000 square feet of surface. This is a spreading rate of 37 square feet per pound of dry glue powder, assuming that the powder is mixed with twice its weight of water. With all other conditions favorable, 65 pounds of wet glue per 1,000 square feet (45 square feet per pound of dry glue mixed 2 to 1) may suffice. The assembly time may be as much as 15 to 20 minutes, but it is usually safer to keep within the lower limit. Under ordinary circumstances good results may be expected with a pressure of 150 to 200 pounds per square inch. For more adequate discussion of the technic of gluing with casein and other glues reference should be made to the publications of Truax.[215]

Time of Pressing

"Casein glues set very quickly and produce joints strong enough to be machined in a few hours. In spruce and woods of similar strength joints as strong as the wood can be expected within 4 hours from the time pressure is applied. In hard maple, which is stronger than spruce, tests showed that about 6 hours were required. It is not necessary to keep the joints under pressure all this time, however. If the pressure is maintained for ½ to 2 hours, depending on the kind of joints, and the glued members are then allowed to season before working, good results may be expected.

"The above statements refer to the minimum time in which good results may be obtained. In case of emergency, it might be safe to machine wood containing casein glue joints as soon as four to six hours after pressure was applied. Under ordinary conditions this is not desirable.

The best practice is to allow glue joints to season[92] until the moisture at the glue line has dried out or distributed itself uniformly throughout the wood. In this way sunken joints[83] can be avoided and the tendency of the glued block to change dimensions or shape after machining will be reduced."[93]

Hot-Press and Dry Gluing Technics

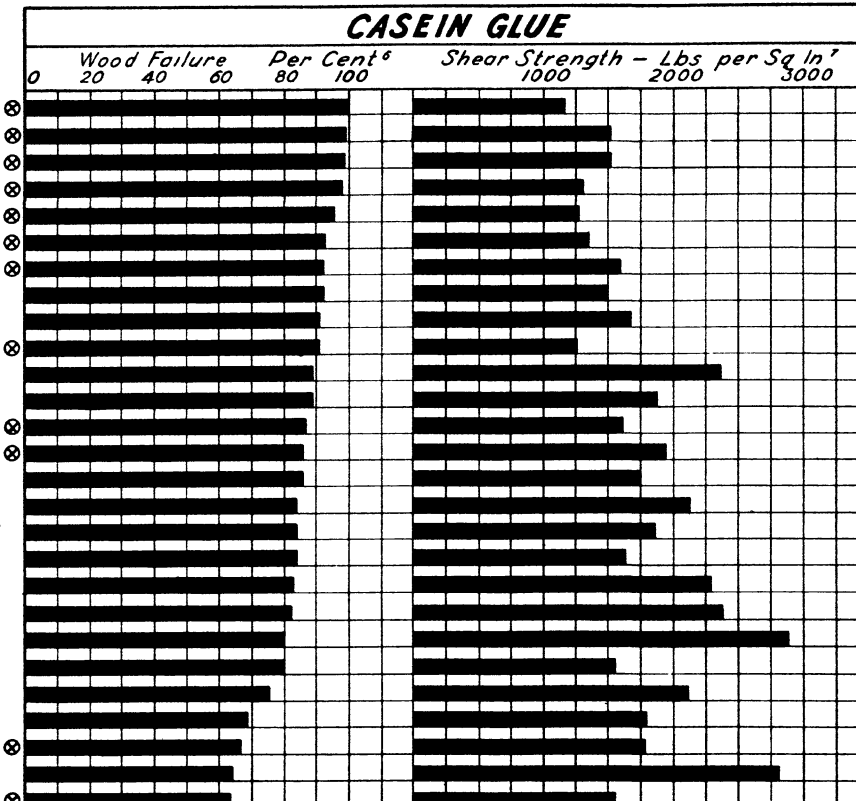
Most gluing in the woodworking industries is done by the cold-press technic, that is, without application of heat at the time of pressing the joint. For making joints between pieces of lumber of substantial thickness the cold-press technic is usually the only practicable one because the low heat conductivity of wood makes it difficult to supply heat to the glue line promptly enough, and undue drying of the wood in the course of the heating gives trouble with shrinking, warping, and checking. In gluing plywood and face veneers, however, the glue line may be heated quickly and the thinness of the veneers permits them to withstand great changes in moisture content without undue damage, so that the hot-press technic becomes practicable.

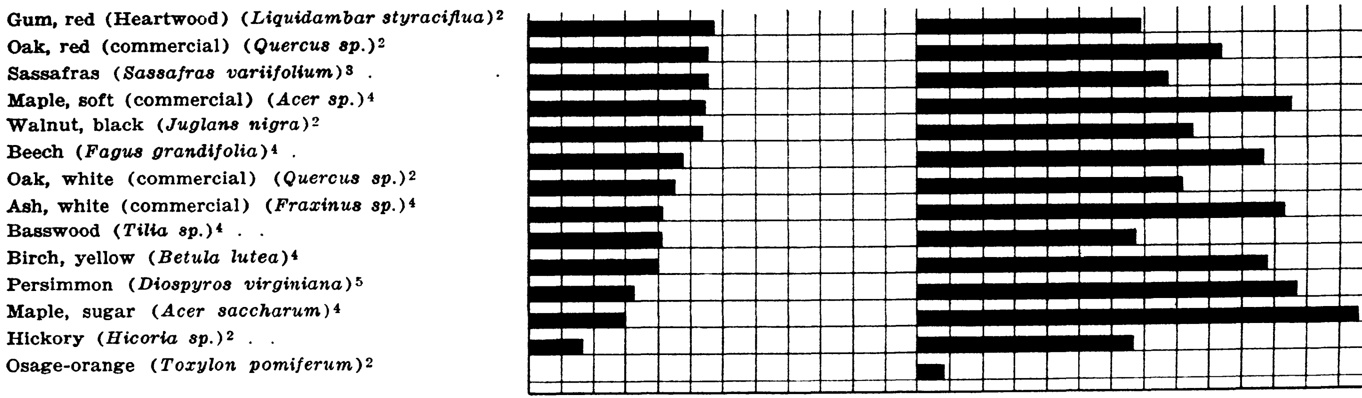
Any of the ordinary casein glues may be used for making plywood by the hot-press technic, in which hydraulic presses with hollow platens usually heated by steam are used. As a rule the water-resistance of the glued joint is materially improved by hot pressing, especially if the glue is one of only moderate water-resistance when used in cold-press methods. Unusual formulations of casein glue for hot pressing are also proposed, such as casein and paraformaldehyde dissolved in a concentrated solution of ammonium thiocyanate.[169] Hot-press gluing with casein glue has long been practiced in Europe[106, 114] but until recent years the woodworking industries in this country generally considered hot-press gluing too expensive in equipment and cost of labor. With the development of the highly water-resistant resin glues, however, hot-press gluing is finding a substantial place in American practice.

With the aid of the hot press it is possible to make plywood by the technic of dry gluing, the object of which is to avoid introducing a relatively large amount of water into the joint to be later removed by passage through the wood. In the earliest process of dry gluing,[90, 206, 216] which was developed with blood glue but could be adapted to casein glue, thin tissue paper or fabric is impregnated with the glue and dried. A hygroscopic material such as sugar is incorporated in the glue, however, so that it will retain a small amount of moisture, which is necessary. A sheet of the prepared glue is placed between the surfaces to be joined and pressure applied for a few minutes in a hot press. The glue may also be spread on one of the surfaces to be joined and allowed to dry before assembling and pressing. This process is particularly applicable to the gluing of veneers from 1/30 to 1/100 inch thick, which curl and

SPECIES[1]

Redwood (*Sequoia sempervirens*)[2] ⊗
Cedar, western red (*Thuja plicata*)[2] ⊗
Fir, white (*Abies concolor*)[4] ⊗
Spruce, Sitka (*Picea sitchensis*)[4] ⊗
Cypress, southern (*Taxodium distichum*)[4] ⊗
Hemlock, western (*Tsuga heterophylla*)[4] ⊗
Cedar, eastern red (*Juniperus virginiana*)[4] ⊗
Chestnut (*Castanea dentata*)[2]
Alder, red (*Alnus rubra*)[2]
Pine, northern white (*Pinus strobus*)[4] ⊗
Magnolia (*Magnolia sp*)[4]
Elm, American (*Ulmus americana*)[4]
Pine, ponderosa (*Pinus ponderosa*)[4] ⊗
Pine, southern yellow (*Pinus sp*)[4] ⊗
Cottonwood (*Populus sp*)[4]
Gum, tupelo (commercial) (*Nyssa sp*)[4]
Gum, red (Sapwood) (*Liquidambar styraciflua*)[5]
Poplar, yellow (*Liriodendron tulipifera*)[2]
Sycamore (*Platanus occidentalis*)[4]
Gum, black (commercial) (*Nyssa sp*)[4]
Elm, rock (*Ulmus racemosa*)[2]
Butternut (*Juglans cinerea*)[2]
Cherry, black (*Prunus serotina*)[2]
Mahogany (*Swietenia sp*)[2]
Douglas fir (*Pseudotsuga taxifolia*)[2] ⊗
Pecan (*Hicoria pecan*)[2]
Cedar, Alaska (*Chamaecyparis nootkatensis*)[4] ⊗





1 Common and scientific names are the standard names given in U. S. Dept. Agr. Misc. Circ. 92 except those designated "(commercial)."
2 Heartwood.
3 Mostly heartwood.
4 Heartwood and sapwood mixed or not identified.
5 Sapwood.
6 Wood failure per cent indicates the estimated proportion of the joint area of the specimen where wood fibers were torn away in testing.
7 The shear strength of joints is not comparable with the shear strength of solid wood, published in U. S. Dept. Agr. Bull. 556 and elsewhere, due to differences in the test methods and specimens used.
⊗ Indicates woods of the softwood or non-porous class; others belong to the hardwood or porous class.

Figure 34. Results of joint strength tests using casein glues on different woods, from data of Truax.[215]

become unmanageable in the ordinary technic of wet gluing, or the gluing of highly figured veneers with irregular grain.[168] It is also possible to strew powdered glue over one of the surfaces to be joined, moisten it with water[56] or with a high-boiling liquid such as phenol or glycerol,[57] assemble, and hot-press. If the casein glue is made with a salt containing much water of crystallization, such as sodium carbonate decahydrate, it is not necessary to moisten the glue powder with water.[105, 141]

Results with Different Woods

Figure 34 shows the very excellent joint strengths obtainable with casein glue on a large number of woods. The tests were made by the block shear test described in a subsequent part of this chapter. The woods are arranged in the order of the average "percentage wood failure" found in a large number of tests, using glue of formula **K** under conditions favorable to the production of strong joints. The percentage wood failure in a test specimen is the proportion of the area of fracture occurring in the wood rather than in the glue line. It is assumed to be a measure of how closely the joint strength obtained approaches the ideal of perfect gluing. There is also shown the average shearing strength at which failure of the joint took place. Inasmuch as the shearing strengths of the woods vary greatly, the percentage wood failure is believed to be a better measure of success in gluing than is the shearing strength.

As might be expected the stronger, more dense woods do not show so high a percentage wood failure as the weaker woods, but the high strength values obtained with the strong woods, usually falling very close to the shearing strength of the wood itself, prove the very satisfactory results that can be obtained with casein glue. On some of the heavier woods, such as maple, birch, and oak, stronger joints are obtained if the wood is brushed with a 10 per cent solution of sodium hydroxide or, in the case of hickory, cherry, and red gum, with 10 per cent milk of lime, and allowed to dry before gluing.[91, 215] Osage orange does not make strong joints with casein glue without such preliminary treatment.

Staining of Wood by Casein Glue

All strongly alkaline glues stain certain species of wood. Casein glues, like other glues that are made with considerable alkali, discolor the wood of oak, maple, and some other species.[37, 84, 93, 106] The discoloration appears along the glue line and on the faces of panels where the glue or the alkali from it has penetrated. Very thin faces stain more quickly than faces made with thicker veneer. To leave panels under pressure for an unnecessarily long time encourages staining. If the panels are pressed for as short a period as is safe, and then separated on stickers and dried at once, the tendency to stain is checked. If the lumber is dry and the

veneer well redried before gluing, the tendency to stain is reduced. The use of dry absorbent cauls between the faces of adjacent panels should also help. A thin glue penetrates more and is likely to cause staining where a thicker glue would not.

Modifications of the usual glue formulas have been proposed to reduce trouble with staining. Addition of wood flour,[69] cellulose,[77] powdered leather,[75] and calcium tannate[178, 180] are said to reduce staining somewhat but are probably of limited effectiveness. By reducing the alkalinity of the glue the difficulty with staining may be lessened somewhat; but it is then usually necessary to reduce the content of calcium hydroxide to get sufficient working life, with consequent loss of water-resistance.[114] Neutral casein glues containing no lime can be made by dissolving casein in alkalies or hydrolyzable salts and then adding organic acids such as benzoic acid, phthalic acid, and salicylic acid[13] but they make weaker joints and lack water-resistance.[141]

Hardness and Dulling of Tools

All glues dull tools, such as planer knives and saws, more rapidly than do any of our native commercial woods, but casein glue seems to be a little more objectionable than others in this respect.[32, 88] Excessively thick layers of glue make the trouble more serious. It is probably helpful when making wet-mix glues to discard the last few drops of the lime-water mixture, which often contains coarse, gritty material. Whenever it is practicable to trim panels or to machine glued joints before the joint has dried completely, the wear on the tools may be lessened. In some cases it is practicable to use tools made of harder steel. Reducing the proportion of calcium hydroxide in water-resistant glues is helpful,[123, 156, 198] but the water-resistance is then impaired.

Durability of Joints Made with Casein Glue

Although dry jellies of casein glue are much harder and more brittle than those of animal glue, joints made with casein glue have been subjected for 40 hours to a high-speed vibration test without showing any loss of strength.[7] Well-glued wood joints made with casein glue or any of the other important woodworking glues last indefinitely under reasonably dry conditions.

Under damp or alternately wet and dry conditions glues last for some time but they gradually lose strength and finally fail. There are three principal causes of eventual failure under damp conditions: (1) chemical hydrolysis of the casein, (2) destruction of the casein or the wood or both by microörganisms, (3) progressive mechanical failure under alternating wet and dry conditions caused by severe stressing of the joint by swelling and shrinking of the wood.

The effect of various conditions of exposure on the rate of deteriora-

tion of joints made with casein glue is illustrated by the data of Figure 35.[85] Specimens of birch plywood, 3/16 inch thick, were exposed to six test conditions and the strength of the joints determined at intervals, the results being expressed in percentages of the strength of the joints when freshly made. The data are averages for three casein glues, one of formula **K** and two of formula **G** with high and low alkalinity respectively. In Test 1 the specimens were kept in a room held at 30 per cent relative humidity; in Test 2 they were kept alternately in 30 and 60 per cent humidity for intervals of 2 weeks; in Test 3 alternately in 30 and 80 per cent humidity; in Test 4 in 30 and 90 per cent humidity; in Test 5 in 30 and 97 per cent humidity; and in Test 6 they were alternately

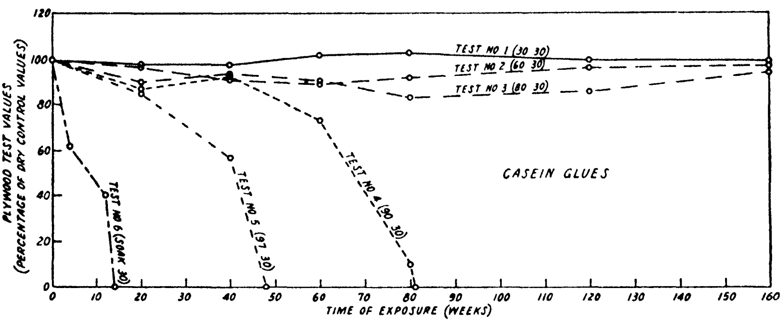


Figure 35.—Rate of deterioration of casein glued joints in plywood under varying conditions of exposure. Test 1, kept constantly in 30 per cent relative humidity; test 2, kept alternately for two weeks in 60 and in 30 per cent humidity; test 3, alternately in 80 and 30 per cent; test 4, in 90 and 30 per cent; test 5, in 97 and 30 per cent; test 6, soaked in water for 2 days and then kept in 30 per cent humidity for 12 days. (Data of Brouse.[85])

soaked in water for two days and kept in 30 per cent humidity for 12 days.

After more than three years in Tests 1, 2 and 3 the specimens made with the casein glues exhibited no loss in strength. Specimens made with animal and vegetable glues withstood Tests 1 and 2 equally well, but in Test 3 they lost strength continuously and failed completely in 80 weeks. In Test 4 specimens made with casein glue remained strong for about a year but failed completely after 80 weeks. Specimens made with hot-pressed blood glue retained nearly 80 per cent of their initial strength after more than three years in Test 4. In Test 5 specimens made with casein glues failed in a little less than a year, whereas those made with blood glue retained 50 per cent of their initial strength after more than 3 years. In Test 6 casein glues failed in 15 weeks, but blood glues retained 60 per cent of the initial strength at that time and did not fail until 120 weeks had passed.

In Tests 4, 5 and 6, the highly alkaline casein glues fail somewhat more rapidly than the glues of low alkalinity because hydrolysis goes on faster in the former.[34] The superiority of glues of low alkalinity is most marked in tests in which the plywood specimens are soaked continuously in water, under which conditions glues of low alkalinity may retain 25 per cent of their original strength after more than two years, whereas strongly alkaline glues may fail completely within a year. Continuous soaking is a much less severe test than the alternate soaking and drying of Test 6 or even the alternation between 30 and 97 per cent humidity of Test 5. Where wood and glue are saturated with water to the exclusion of air, fungi are not active and progressive mechanical failure is less serious than in Tests 5 and 6.

When glued joints are exposed continuously to 97 per cent relative humidity at 80° F., both wood and glue are actively attacked by fungi, hydrolysis is somewhat less rapid than in the soaking test, and progressive mechanical failure is not particularly severe. Under such conditions the performance of casein glues can be greatly improved by incorporating preservative chemicals in the glue or, still better, by impregnating the plywood with preservatives after gluing.[33,124] Substantial proportions of preservative, however, are required. Beta naphthol in proportions of 10 parts by weight to 100 parts of casein, and creosote in proportions of 20 parts, are effective additions to the glue, and impregnation of the plywood after gluing with creosote or with a 25 per cent solution of beta naphthol in linseed oil is also effective. On the other hand, coating the plywood with asphalt paint or with aluminum paint was found to be of no value when the specimens were exposed continuously to dampness. If the specimens are exposed to dampness only for short intervals, as would be the case with plywood exposed to the weather, moisture retardant coatings are effective provided the coatings are kept in good condition. The edges of the plywood are then the critical points where protection must be most carefully maintained and where it is most difficult to do so.

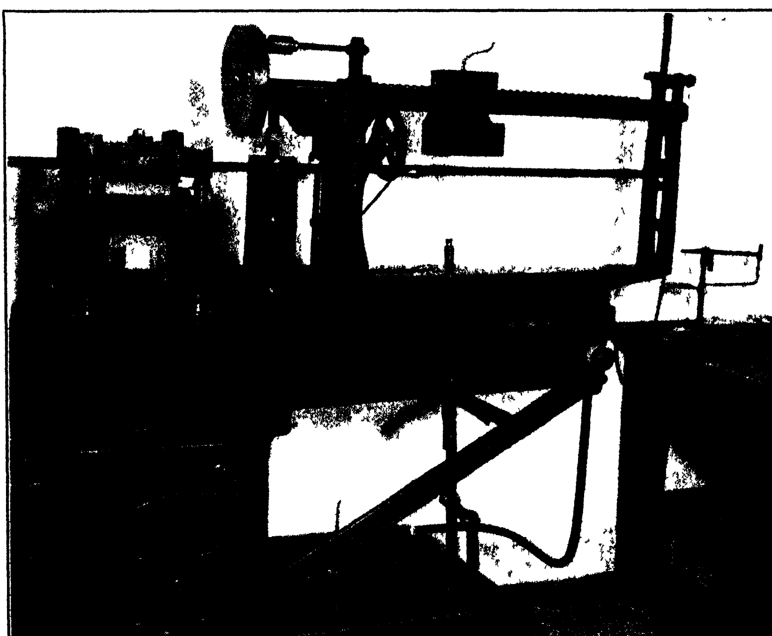
Testing Casein Glue

Acceptance tests for incorporation in specifications for the purchase of casein glue have so far been confined largely to a test of the strength of dry joints and a plywood water-resistance test. The strength of a glued wood joint in general depends as much upon the strength of the wood and the conditions under which the gluing is done as it does on the properties of the glue;[214, 215] consequently tests of joint strength require wisely chosen and carefully controlled technic followed by intelligent interpretation. Such tests do not measure the strength of adhesion of the glue, for well-glued joints are sometimes stronger than films of the glue with which they are made.[40] Tests of joint strength do show

whether the glue is capable of meeting minimum standards of performance under the particular conditions chosen for the test.

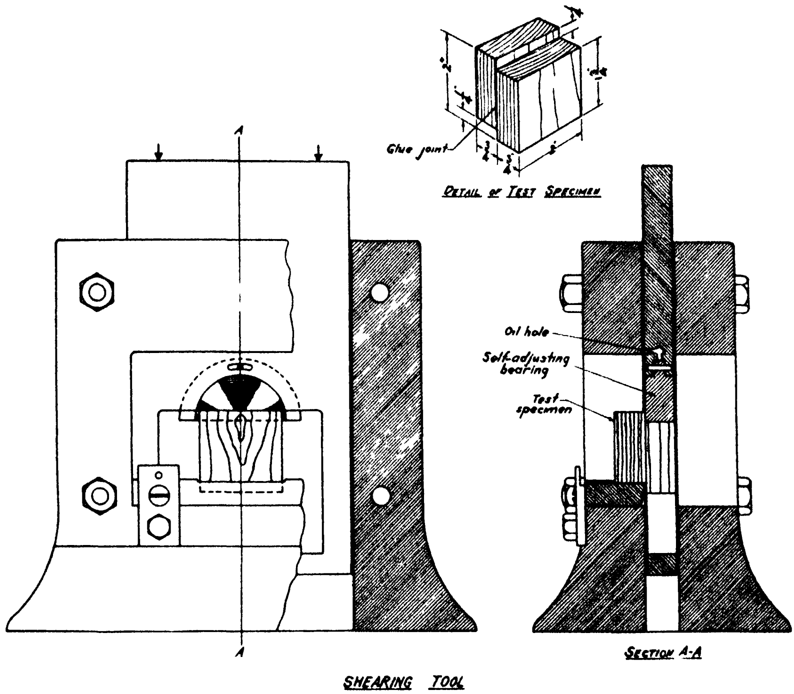
The Joint Strength Test

The joint strength test, as developed at the Forest Products Laboratory, is made as follows: ¾-inch hard maple lumber, free from defects and selected for straight grain and high density, is thoroughly seasoned by storage in a room at 80°F. and 30 per cent relative humidity. Just before required for a test, specimens either 2½ inches by 12 inches or 5 inches by 12 inches are cut and the surfaces to be joined are planed smooth and matched carefully. (The grain of the wood in each block runs parallel to the long dimension.) The glue, mixed exactly in accordance with the manufacturer's directions, is applied to one of the surfaces to be joined, the quantity of glue spread being determined by weighing the block before and after application. The two surfaces are brought together, a 5- to 10-minute assembly time permitted to elapse; then the block is placed in the press and a pressure of 200 pounds per square inch applied. The block is left in the press over night and then



Courtesy of the Forest Products Laboratory

Figure 36A.—Apparatus for making the joint strength test.



Courtesy of the Forest Products Laboratory

Figure 36B.—Detail of test specimen and shearing tool used in making the joint strength test.

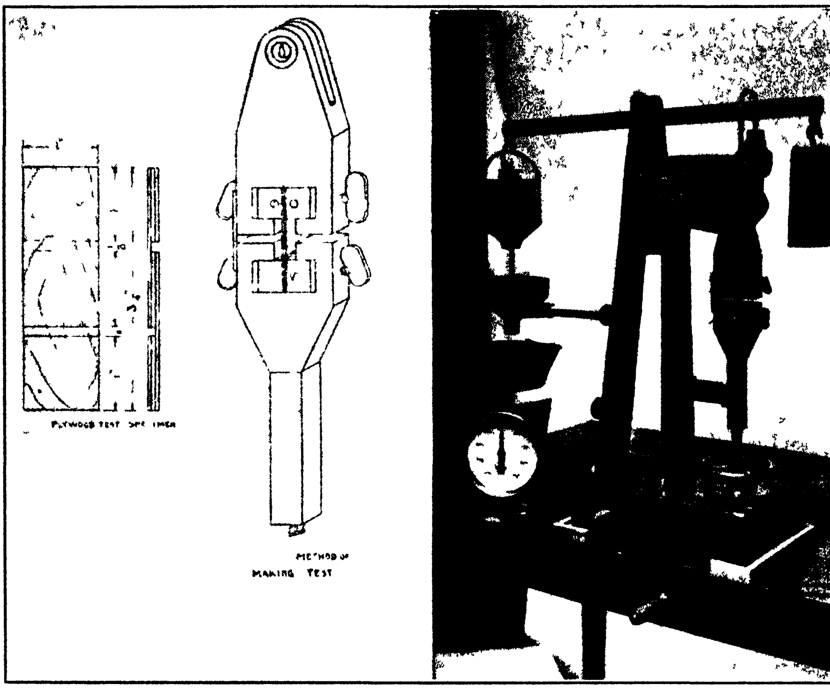
removed to the 30 per cent humidity room for seasoning for 7 to 10 days. It is then cut into 5 or 10 test specimens of the dimensions shown in Figure 36B and the specimens tested, using the special shearing tool illustrated.

The load at which failure takes place, divided by the measured area of the glue line of the specimen, is recorded as the strength in shear. The specimen is examined and an estimate made of the percentage of the area of failure occurring in the wood rather than in the glue line. The averages of the results with all the specimens from at least eight test blocks are taken as representative of the glue when used under the gluing conditions followed in the test.

The Water-resistance Test

One-sixteenth-inch birch veneer, selected for straight grain and freedom from defects, is cut into 12- by 12-inch size and stored in a room at 80°F. and 65 per cent relative humidity until required for use.

A test panel is made by gluing three of these together with the grain of the corepiece at right angles to that of the face plies. The glue, mixed in accordance with the manufacturer's directions, is spread on both sides of the corepiece and the amount determined by weighing the three pieces of veneer before and after application. A closed assembly time of 5 to 10 minutes is allowed to elapse before applying pressure to the panel.



Courtesy of the Forest Products Laboratory

Figure 37.—Apparatus and test specimen used for making the plywood water-resistance test.

A pressure of 200 pounds per square inch is then applied and the panel left in the press over night.

Next day the test panel, together with other similar ones, is stacked on stickers in the 65 per cent humidity room for 7 to 10 days for seasoning. Test specimens of the dimensions shown in Figure 37 are then cut, five of which are tested dry and five submerged in water for two days, after which they are tested while still wet.

The test is made under tension in the cement testing machine with the special jaws illustrated. The load at failure is recorded and the percentage wood failure estimated, the averages for the five dry and five

wet specimens respectively being taken as representative of the panel. At least eight such panels are made and tested and the average results for the eight panels taken as representative of the glue when used under the gluing conditions followed in the test.

Uses of Casein Glue

In the Woodworking Industries

Casein glue is used principally in the woodworking industries. There are, however, many minor uses in other industries about which it is difficult to collect information. In all its uses it is in keen competition with other glues.[89, 146] In cost it is more expensive than vegetable glue and soybean glue but cheaper than animal glue, blood albumin glue, or resin glues. Casein glue has the advantage over vegetable glue in being easily made water-resistant and usually of setting more rapidly after a joint is made. While soybean glues are also water-resistant they are not as satisfactory as casein glues for making strong joints with the denser hardwoods. Animal glues are particularly adaptable to gluing operations with short assembly periods and to the gluing of thin veneers with fancy grain where penetration of an alkaline glue causes trouble with staining. In the form of liquid glue, animal and fish glues are convenient for repair work and household use, where small quantities are required at unpredictable intervals. Casein glue, however, is also marketed through retail stores in small packages for similar use. Blood albumin glue is somewhat more water-resistant than casein glue but the highly water-resistant formulas require hot pressing, which limits their use to plywood and veneered panels. Where the highest degree of water-resistance is required, synthetic resin glues of the phenol-formaldehyde[29, 140, 166, 209] and urea-formaldehyde[58, 166] types are now coming into extensive use. For maximum water-resistance, the resin glues appear to require hot pressing, but efforts are being made constantly to develop resin glues of high quality for cold pressing.[5] In general, casein glue is chosen when a reasonable degree of water-resistance is necessary and sufficient, or where water-resistance is required and hot pressing is impracticable.

In the furniture industry casein glue is widely used for making plywood and veneered panels and for joint work whenever greater water-resistance than that provided by vegetable and animal glue is considered necessary. Wherever the product may be exposed for appreciable periods of time to relative humidities greater than 80 per cent, water-resistant glue is advisable. Furniture for export, particularly to the tropics, and for use in some parts of the South may well be made with casein glue. Furniture and toys to be used at times out of doors but with adequate protective coatings and shelter from the more severe weather are suitably made with casein glue. Casein glue also finds favor in the difficult gluing

operations in making laminated bentwork, such as the rims of grand pianos.

Aircraft manufacture, which gave rise to the development of casein glues in the United States, continues to require it in substantial quantities, particularly for small commercial and pleasure aircraft. Aircraft plywood, however, which can be hot pressed, is usually made with resin glues.

Casein glue was once widely used in making wooden autobodies, which now have been largely superseded by metal. Floor boards and trunk shelves, however, are commonly of plywood glued either with casein or soybean glue. Wooden bodies for trucks likewise consume much casein glue.

Plymetal, which is plywood with sheet metal glued on one or both faces, is commonly made with casein glue. Wood veneer is also glued with casein glue on the surface of metal fire doors, elevator cabs, and other places where fireproof construction is required but the decorative effect of wood is desired. Where fire resistance is obtained by impregnating wood with chemicals, casein glue must be used with discrimination because salts of acid reaction or ammonium salts in the wood may react with casein glue and impair the joint.

Casein glue finds numerous uses in the manufacture of sporting goods, for example, in building up the laminations in tennis racquets. In manufacturing baseball bats and tool handles the wood is sometimes impregnated to some depth with casein glue under pressure and subsequently treated with formaldehyde for the purpose of preventing "splitting of the wood on its surface and to render the surface waterproof, so that swelling will be prevented or greatly retarded."[155, 226] As a "waterproofing" in this sense, casein glue can hardly be expected to prove highly effective.

Large quantities of casein glue are consumed in the manufacture of trunks and hand baggage, both for the plywood that is now being used very extensively and for gluing the members together and covering the surfaces with fabric or leather.

There are many other woodworking industries, large and small, in which casein glue has made a place for itself, from butchers' blocks and refrigerators to wooden pulleys. In some industries, as in casket manufacture, casein glue may be used largely for the sake of providing the sales argument that the product is made with water-resistant glue.

Casein and soybean glues find a large and increasing use in the construction industries. They are used for structural plywood, such as plywood for house sheathing and subflooring, and for interior plywood, such as wall and ceiling panels and panels for doors, cabinets, and store fixtures. Plywood for concrete forms is appropriately made of casein or soybean glue unless the conditions of service are such that the panels may

be used an unusually large number of times, justifying the added cost of resin-glued plywood.

Casein glue has become thoroughly established in the manufacture of millwork, where it is used for doors, built-in cabinets, stair parts, and



Figure 38.—Use of glued wood construction in garage and storage shed at the Forest Products Laboratory, Madison, Wisconsin. Supporting arches are of laminated wood glued with casein glue. Interior walls are covered with plywood glued with soybean glue. Exterior walls are covered with plywood glued with resin glue and painted after erection.

other items. Since woodwork going into new buildings is commonly subject for a time to dampness, use of a moderately water-resistant glue is desirable.

Most gluing in connection with the construction industries is done in plywood or millwork factories supplying units to be assembled on the

job. In general the technic of gluing is too exacting to be done adequately and economically by hand. These are times of experimentation in construction methods, however, and some proposals do involve gluing on the

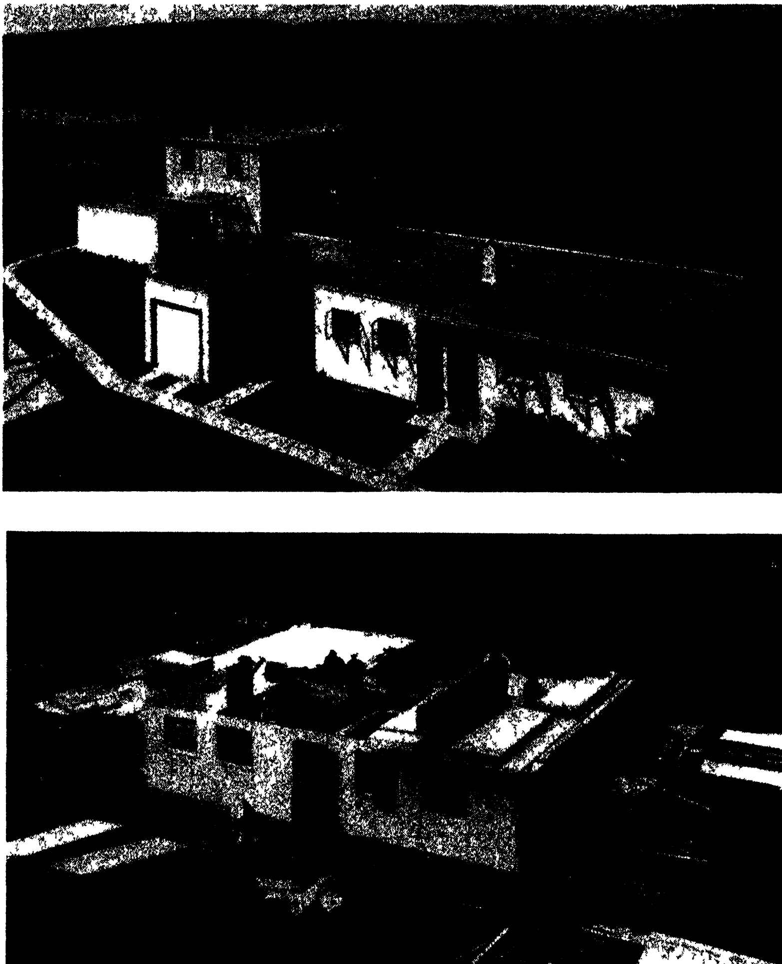


Figure 39—Experimental prefabricated houses erected by the Forest Products Laboratory, making use of wall, floor, and roof panels consisting of plywood glued with casein glue to an inner structural framework

job with casein glue. Gluing of plywood wall covering directly to studs,[60] joining of plywood wall panels by gluing splines at the joints,[49] and joining prefabricated wall units with glue[4] have been seriously advocated.

Glue as a method of fastening in wood construction may well become more important in the future than it has been in the past but it will probably come about by increased use of glue in those items that can be made more economically in the factory rather than in gluing on the job. Figure 38 illustrates several forms of laminated arches made of wood glued with casein glue. Construction of this kind makes it possible to use wood economically for structural support over large spans. Large laminated columns, beams, and arches can be made of kiln-dried wood, all of which can be inspected thoroughly for moisture content and defects; and select and common grades can be combined within the members in such a way as to achieve satisfactory utilization of the lumber without loss in strength from knots and other defects. The building illustrated is primarily an experimental one, but laminated structural members of the kind are now in commercial production in the United States. They have been used in Europe for some years.

Figure 39 shows the construction of two experimental houses making use of prefabricated wall, floor, and roof panels in which gluing plays an important part.[151] Each panel consists of two plywood faces glued to an inner structural framework to form what is virtually a box girder. Because of the gluing of the plywood to the framework, the plywood carries a material proportion of the load and the framework may be made of smaller pieces of wood than the studs and joists of conventional frame construction. The plywood is made with resin glue but the plywood is glued to the framework with casein glue.

Other Uses of Casein Glue

Casein glue finds many applications outside of the woodworking industries. Many of them are unknown to the writers. In practically all of them casein competes with other glues. The following examples are given merely for the purpose of illustrating uses for which casein glue has been found suitable at one time or another.

Casein glue may be used for attaching linoleum to wood floors or table tops. When automobile running boards were made of linoleum glued to wood this was said to be the second largest market for casein glue.

In making laminated safety glass, casein glue was early recognized as one of the adhesives suitable for joining glass to celluloid[45, 161, 191, 221, 222] and has been used commercially for that purpose. Laminated safety glass usually consists of two pieces of plate glass glued on either side of a sheet of plastic, such as celluloid or other cellulose ester plastic. Because glass is practically impermeable to water, the gluing must be done by a method that is essentially dry gluing.[16] The glue, which may be an aqueous solution containing 3 per cent casein and 0.45 per cent borax, to which a polyglycol may be added,[219] is applied to the cleansed surface of the glass and nearly all the water allowed to evaporate; the "sandwich"

is then assembled and hot pressed by means of suitable equipment. Casein may also be used as a modifying agent in a resin adhesive for cellulose ester plastics.[50] According to patent claims, casein plastic may be used for the central lamination in safety glass.[17, 44]

For gluing paper, cardboard, wood, glass, or metal, casein glues are suitable and find many minor uses. In the manufacture of cigarettes, casein glues mixed with a minimum of water make seams that do not wrinkle and remain firm when exposed to humid conditions. At the agricultural experiment stations of several universities, water-resistant casein glues have been found useful for fastening paper bags over the mature flowers of plants to prevent uncontrolled pollination in breeding studies. They have been used for sealing paper bottles and cartons[12, 134, 145] and for pasting labels on glassware and metal containers. In the latter case the glue usually is made with casein, an alkali, and sodium resinate.[12]

Casein glue has long been used very widely as a sizing material.[28, 63, 72, 80, 82, 108, 115, 116, 121, 129, 132, 164, 170, 190, 194, 207, 210, 218, 224] Casein sizings are used on such diverse products as shot-gun shells and heels for ladies' shoes. The casein varnishes discussed in Chapter 10 often serve more as sizings than as coatings. Casein sizing in paper making is discussed in Chapter 9, in leather finishing in Chapter 11, and in textile manufacture in Chapter 13.

Casein glue enters into the manufacture of various kinds of composition materials as a binder. Composition cork, for example, may be made by mixing casein with water containing borax or other solvents and glycerol or glycerol substitutes, melting the mixture in steam-jacketed kettles, adding an insolubilizing agent, then spraying the mixture over the granulated cork and molding the mass in metal molds heated to the temperature of boiling water and cooling the molds before removing the product. Resins or drying oils may be incorporated with the casein binder.[96a] Casein glue may also be used to fasten cork or paper discs to metal shells in the bottle-cap industry. According to patent claims, casein is a suitable binder for such composition materials as tiles, mats, billiard balls, and linoleum substitutes[20] and for making abrasive-coated paper or fabric for grinding and polishing.[18]

References

1. Aktiebolaget Lauxein-Casco, British Pat. 376,748 (July 13, 1932)
2. Anonymous, "Contreplaqués et colles à la caséine," Société de Publications Mécaniques, Paris, 1928
3. Anonymous, *Industrial Finishing Mag. (London)*, **1**, 184 (1930)
4. Anonymous, *Amer. Builder and Building Age*, **59**, 80 (July, 1937)
5. Anonymous, *Hardwood Record*, **76**, 24 (Jan. 1938)
6. Anonymous, *Paint Oil & Chem. Rev.*, **100**, No. 10, 45 (1938)

7. Araki, T. and Matsuno, T., *J. Soc. Chem. Ind. Japan,* **32,** 591, 595, *Suppl. Binding,* **183,** 184B (1929)
8. Bacharov, N. F., *Trans. Central Aero-Hydrodynam. Inst. (U.S.S.R.),* No. 115 (1932) ; *Chem. Zentr.,* **1933I,** 3858
9. Bateman, E., and Town, G. G., *Ind. Eng. Chem.,* **15,** 371 (1923)
10. Battline, F., *Paint Oil & Chem. Rev.,* **100,** No. 9, 9 (1938)
11. Bauer, R. and Mauthe, G., German Pat. 574,841 (Oct. 18, 1933) ; French Pat. 755,676 (Nov. 28, 1933)
12. Becher, C., Jr., *Gelatine Leim Klebstoffe,* **3,** 163 (1935)
13. Becher, C., Jr., *Gelatine Leim Klebstoffe,* **5,** 147 (1937)
14. Behnke, P. H., British Pat. 314,344 (June 25, 1928)
15. Bellamy, C. H., U. S. Pat. 681,436 (Aug. 27, 1901)
16. Benedictus, E., U. S. Pat. 1,182,739 (May 9, 1916) ; British Pat. 18,856 (Nov. 30, 1911) ; see also *Chem. & Met. Eng.,* **39,** 266 (1932)
17. Benedictus, E., U. S. Pat. 1,098,342 (May 26, 1914) ; 1,128,094 (Feb. 9, 1915) ; *Glaces et verres,* No. 18, 9 (1930)
18. Benner, R. C. and Melton, R. L., British Pat. 462,696 and 462,697 Abridg. Spec. 1937 Group IV, 93, Group V, 93
19. Bernstein, A., U. S. Pat. 845,681 (Feb. 26, 1907)
20. Biddle, A., U. S. Pat. 1,437,487 (Dec. 5, 1922) ; Reissues 16,476 and 16,477 (Nov. 16, 1926) ; British Pat. 253,740; U. S. Pat. 1,777,157, 1,777,161, 1,777,-162 (Sept. 30, 1930)
21. Bogue, R. H., "Chemistry and Technology of Gelatin and Glue," New York, McGraw-Hill Publishing Co., 1922
22. Brabrook, G. H., U. S. Pat. 1,244,463 (Oct. 30, 1917)
23. Bradshaw, L., U. S. Pat. 1,787,611 (Jan. 6, 1931)
24. Bradshaw, L., U. S. Pat. 1,833,771, 1,833,772 (Nov. 24, 1931)
25. Bradshaw, L., U. S. Pat. 1,895,433 (Jan. 24, 1933)
26. Bradshaw, L. and Dunham, H. V., U. S. Pat. 1,703,134 (Feb. 26, 1929)
27. Bradshaw, L. and Dunham, H. V., U. S. Pat. 1,829,258, 1,829,259 (Oct. 27, 1931)
28. Bremer, H., U. S. Pat. 650,003 (May 22, 1900)
29. Brenner, P. and Kraemer, O., "Fachausschuss für Holzfragen" (Berlin) Publication No. 12 (1935)
30. Brooks, H. K., U. S. Pat. 750,048 (Jan. 19. 1904)
31. Broquette, C. A., U. S. Pat. 8,035 (Apr. 15, 1851)
32. Brouse, D., *Wood Working Industries,* **2,** No. 3, 23 (1926)
33. Brouse, D., *Mech. Eng.,* **53,** 664 (1931) ; *Wood Working Industries,* **9,** No. 6, 32 (1931) ; **11,** No. 2, 30 (1932) ; *Furniture Mfr.,* **42,** No. 1, 57 (1931) ; **43,** No. 6, 33 (1932) ; *Hardwood Record,* **69,** No. 8, 39 (1931) ; *Wood Products,* **9,** No. 8, 19 (1931) ; Forest Products Labty. mimeograph R1125
34. Brouse, D., *Furniture Mfr.,* **48,** No. 3, 9 (1934) ; *Wood Products,* **12,** No. 10, 8 (1934) ; Forest Products Labty. mimeograph R1050
35. Brouse, D., *Mech. Eng.,* **60,** 306 (1938) ; Forest Products Labty. mimeograph R1172; paper (in press) before Amer. Soc. Mech. Eng., Wood Industries Div., High Point, N. C., Sept. 1938
36. Browne, F. L., *J. Ind. Eng. Chem.,* **11,** 1019 (1919)
37. Browne, F. L., *Veneers* (Apr., May 1928)
38. Browne, F. L., *Ind. Eng. Chem.,* **23,** 290 (1931) ; *Paint Oil & Chem. Rev.,* **100,** No. 12, 28 (1938)
39. Browne, F. L. and Brouse, D., "Colloid Symposium Monograph," **5,** 229, New York, Reinhold Pub. Corp., 1928
40. Browne, F. L. and Brouse, D., *Ind. Eng. Chem.,* **21,** 80 (1929)

41. Browne, F. L., Brouse, D., and Hrubesky, C. E., "Chemical and Physical Studies of Water-Resistant Glues," Forest Products Labty. Project 157 (1929)
42. Browne, F. L. and Hrubesky, C. E., *Ind. Eng. Chem.*, **19**, 215 (1927); Browne, U. S. Pat. 1,712,077 (May 7, 1929)
43. Browne, F. L. and Truax, T. R., "Colloid Symposium Monograph," **4**, 258, New York, Reinhold Pub. Corp., 1926
44. Bull, U. S. Pat. 1,539,239 (May 26, 1925); 1,553,667 (Sept. 15, 1925)
45. Bull, U. S. Pat. 1,632,363 (June 14, 1927)
46. Butterman, S., U. S. Pat. 1,291,396 (Jan. 14, 1919)
47. Butterman, S., *J. Ind. Eng. Chem.*, **12**, 141 (1920)
48. Butterman, S., and Cooperrider, C. K., U. S. Pat. 1,456,842 (May 29, 1923)
49. Campbell, C. C., *Veneers and Plywood*, **30**, No. 10, 8 (1936)
50. Charch, W. H. and Snyder, J. E., U. S. Pat. 1,929,013 (Oct. 3, 1932)
51. Christopher, E. F., U. S. Pat. 1,962,763 (June 12, 1934)
52. Dance, E. L., British Pat. 187,200 (Apr. 28, 1922); U. S. Pat. 1,478,943 (Dec. 25, 1923)
53. Davidson, G. and Laucks, I. F., U. S. Pat. 1,691,661 (Nov. 13, 1928)
54. Davies, W. L., *Ind. Chemist*, **5**, 202 (1929)
55. De Beukalaer, F. L., Powell, J. R., and Bahlmann, E. F., *Ind. Eng. Chem.*, **16**, 310 (1924)
56. Dike, T. W., U. S. Pat. 1,922,668
57. Dike, T. W., U. S. Pat. 1,923,922
58. Dike, T. W., *Ind. Eng. Chem., News Ed.*, **14**, 378 (1936); *Veneers*, (July, 1934)
59. Douglas, W. D. and Pettifor, C. B., *J. Roy. Aeronautical Soc.*, **33**, 92 (1929)
60. Douglas Fir Plywood Assoc., Tacoma, Wash., "Specifications and Suggestions for Interior Paint Decoration on Douglas Fir Plywood."
61. Dreyfus, L. A., U. S. Pat. 759,808 (1904)
62. Drushel, U. S. Pat. 1,473,987 (Nov. 13, 1922)
63. Dunham, A. A., U. S. Pat. 739,657 (Sept. 22, 1903); 1,141,951 (June 8, 1915)
64. Dunham, A. A., U. S. Pat. 1,373,224 (Mar. 29, 1921)
65. Dunham, A. A., U. S. Pat. 1,391,768 (Sept. 27, 1921)
66. Dunham, A. A., U. S. Pat. 1,391,769 (Sept. 27, 1921)
67. Dunham, A. A., U. S. Pat. 1,391,770 (Sept. 27, 1921)
68. Dunham, A. A., U. S. Pat. 1,537,939 (May 19, 1925)
69. Dunham, A. A., U. S. Pat. 1,571,662 (Feb. 2, 1926)
70. Dunham, H. V., U. S. Pat. 695,198 (1902); 748,709 (1904)
71. Dunham, H. V., U. S. Pat. 717,085 (Dec. 30, 1902)
72. Dunham, H. V., U. S. Pat. 814,594 (Mar. 6, 1906); 897,885 (Sept. 8, 1908)
73. Dunham, H. V., U. S. Pat. 821,620 (1906)
74. Dunham, H. V., U. S. Pat. 1,347,845 (July 27, 1920); British Pat. 164,604 (May 17, 1920)
75. Dunham, H. V., U. S. Pat. 1,653,551 (Dec. 20, 1927)
76. Dunham, H. V., U. S. Pat. 1,819,878, 1,819,879, 1,819,880 (Aug. 18, 1931)
77. Dunham, H. V., U. S. Pat. 1,892,486 (Dec. 27, 1932)
78. Dunham, H. V., U. S. Pat. 1,895,979 (Jan. 31, 1933)
79. Dunham, H. V., U. S. Pat. 1,971,522 (Aug. 28, 1934)
80. Fargas-Oliva, P., U. S. Pat. 768,274 (Aug. 23, 1904)
81. Federal Specifications Executive Committee, Specification C-G-451 for Animal Glue, May 26, 1931; see also U. S. Navy Dept. Spec. 52G8b (July 1, 1932) for Casein Glue
82. Ferney, D. F., U. S. Pat. 1,235,784 (Aug. 7, 1917)

83. Forest Products Laboratory, Madison, Wisconsin, "Sunken Joints in Furniture Panels," Technical Note 139 (1920)
84. Forest Products Laboratory, "Occurrence and Removal of Glue Stains," Technical Note 146 (1921)
85. Forest Products Laboratory, "Copper Salts Improve Casein Glue," Technical Note 170 (1922)
86. Forest Products Laboratory, "Starved Joints," Technical Note 193 (1923)
87. Forest Products Laboratory, "Strong and Weak Glue Joints," Technical Note 211 (1925)
88. Forest Products Laboratory, "A Factory Method of Testing Hardness of Glued Joints," Technical Note 223 (1926)
89. Forest Products Laboratory, "Glues for Use with Wood," Technical Note 207 (1929)
90. Forest Products Laboratory, "A Dry Glue Method of Laying Veneers," mimeograph R914 (Oct. 1930)
91. Forest Products Laboratory, "Chemical Treatment of Surfaces Improves Glue Joints in Certain Woods," Technical Note 232 (1930)
92. Forest Products Laboratory, "Drying and Conditioning Glued Joints," mimeograph R475 (1935)
93. Forest Products Laboratory, "Casein Glues: Their Manufacture, Preparation and Application," mimeograph R280 (1935)
94. Forest Products Laboratory, "Wood Handbook," Supt. of Documents, Washington, D. C. (1935)
95. Freeman, J., British Pat. 248,990 (July 7, 1925)
96. French Pat. 737,578 (Sept. 19, 1931)
96a. Frink, N. S., McManus, C. E., and Weisenburg, A., U. S. Pat. 2,121,791, 2,121,809, 2,121,810, 2,121,844 (June 28, 1938)
97. Gardner, H., British Pat. 21,774 (Mar. 9, 1897)
98. German Pat. 270,200, quoted by Hadert, ref. 113
99. German Pat. 345,684, " " " " "
100. German Pat. 406,401, " " " " "
101. German Pat. 424,745, " " " " "
102. German Pat. 505,033, " " " " "
103. German Pat. 542,442, " " " " "
104. German Pat. 548,395, " " " " "
105. Gerngross, O. and Sandor, G., German Pat. 568,310 (Nov. 21, 1928)
106. Gerngross, O., *Gelatine Leim Klebstoffe*, **1**, 17, 32 (1933)
107. Gerry, E. and Truax, T. R., *Furniture Mfr. & Artisan,* 49 (April, 1922); *Scientific American* (Aug. 1923)
108. Goldsmith, B. B., U. S. Pat. 615,446 (Dec. 6, 1898)
109. Govers, F. X., U. S. Pat. 799,599 (Sept. 12, 1905)
110. Gruendler, G. J., U. S. Pat. 633,834 (Sept. 26, 1899)
111. Grune, W., U. S. Pat. 132,659 (Oct. 29, 1872)
112. Hadert, H., "Kaseinleim und Kaseinfarbenindemittal," Berlin, 1934; "Casein and Its Uses." New York, Chemical Publishing Co., 1938
113. Hadert, H., *Gelatine Leim Klebstoffe,* **2**, 53 (1934)
114. Hadert, H., *Gelatine Leim Klebstoffe,* **5**, 154, 179 (1937)
115. Hall, W. A., U. S. Pat. 609,200 (Aug. 16, 1898)
116. Hall, W. A., U. S. Pat. 623,541 (Apr. 29, 1899); 664,318 (Dec. 18, 1900); 695,926 (Mar. 25, 1902); 758,064 (1904)
117. Hall, W. A., U. S. Pat. 684,545 (Oct. 15, 1901)
118. Hall, W. A., U. S. Pat. 695,927 (Mar. 25, 1902)
119. Hardy, W. B., *Proc. Roy. Soc. (London),* **A100**, 564 (1922)

120. Harvey, M. T., U. S. Pat. 1,952,213 (Mar. 27, 1934)
121. Haynes, T. A., U. S. Pat. 619,040 (Feb. 7, 1899); 684,985 (Oct. 22, 1901)
122. Heim, R., U. S. Pat. 1,018,559 (Feb. 27, 1912)
123. Henning, S. B., Canadian Pat. 226,535 (Nov. 28, 1922)
124. Hermann, A., *Gelatine Leim Klebstoffe,* **2,** 224 (1934); *Sperrholz,* **5,** 166 (1933)
125. Hermann, A., *Kunstdünger und Leim,* **30,** 40 (1933)
126. Hewitt, P. C., U. S. Pat. 522,831 (July 10, 1894)
127. Hirsh, J., U. S. Pat. 86,398 (Feb. 2, 1869)
128. Hoffman, W. F. and Gortner, R. A., "Colloid Symposium Monograph," **2,** 264, New York, Reinhold Publishing Corp., 1925
129. Isaacs, M. R., U. S. Pat. 838,785 (Dec. 18, 1906); 959,348 (May 24, 1910)
130. Isaacs, M. R., U. S. Pat. 845,790 (Mar. 5, 1907); 848,746 (Apr. 2, 1907)
131. Isaacs, M. R., U. S. Pat. 845,791 (Mar. 5, 1907)
132. Isaacs, M. R., U. S. Pat. 879,967 (Feb. 25, 1908); 1,063,974 (June 10, 1913)
133. Isaacs, M. R., U. S. Pat. 1,192,783 (July 25, 1916); 1,603,696 (Oct. 19, 1926)
134. Jovignot, C., U. S. Pat. 920,959 (May 11, 1909)
135. Just, J. A., U. S. Pat. 670,372 (Mar. 19, 1901); 682,549 (Sept. 10, 1901); 692,454 (Feb. 4, 1902); 868,445 (Oct. 15, 1907)
136. Just, J. A., U. S. Pat. 692,450 (Feb. 4, 1902)
137. Kimberlin, W. M., U. S. Pat. 725,094 (Apr. 14, 1903)
138. Knorr, L., British Pat. 148,217 (July 9, 1920)
139. Knorr, L., British Pat. 148,216 (July 28, 1921)
140. Kraemer, O., *Aircraft Engineering,* **10,** 183 (June, 1938); *Modern Plastics,* **15,** No. 12, 42 (1938)
141. Kustner, P., *Gelatine Leim Klebstoffe,* **2,** 254 (1934)
142. Lanzendorfer, G., U. S. Pat. 1,659,271 (Feb. 14, 1928)
143. Laucks, I. F. and Davidson, G., U. S. Pat. 1,786,209 (Dec. 23, 1930); Brown, E. D., Davidson and Laucks, U. S. Pat. 1,836,897 (Dec. 15, 1931)
144. Lee, W. B., *Ind. Eng. Chem.,* **22,** 778 (1930)
145. Leibfarth, R., British Pat. 362,011 (Sept. 10, 1930)
146. Leicester, W. F., *Amer. Soc. Mech. Engrs., Sixth Annual Wood Industries Meeting,* Winston-Salem, N. C., Oct. 15, 16 (1931)
147. Leicester, W. F., U. S. Pat. 1,873,827 (Aug. 23, 1932); 2,028,287.
148. Lindauer, A., U. S. Pat. 1,310,706 (July 22, 1919)
149. Lindauer, A. and Hunt, G. M., U. S. Pat. 1,506,013 (1924)
150. Lindstaedt, F. F., U. S. Pat. 1,833,527 (Nov. 24, 1931)
151. Luxford, R. F., *Timberman,* **39,** No. 2 *(Plywood Supplement)* 10 (1937)
152. Majert, W., U. S. Pat. 586,854 (July 20, 1897)
153. Markwardt, L. J. and Wilson, T. R. C., *U. S. Dept. of Agric., Technical Bulletin* 479 (Sept., 1935)
154. Marotta, D., *Ann. chim. applicata,* **6,** 165 (1916)
155. Martin, G., "Industrial and Manufacturing Chemistry," 6th ed., part 1, p. 527, London, Crosby Lockwood, 1922
156. Marwin, A. C., U. S. Pat. 1,625,483 (Apr. 19, 1927)
157. McBain, J. W. and Hopkins, D. G., *J. Phys. Chem.,* **29,** 188 (1925); **30, 114** (1926), *Dept. of Scientific and Industrial Research (Gt. Britain), Adhesives Research Comm.,* **2,** 34 (1926)
158. McBain, J. W. and Lee, W. B., *J. Soc. Chem. Ind.,* **46,** 321 (1927); *J. Phys. Chem.,* **31,** 1647 (1927); **32,** 1178 (1928); *Proc. Roy. Soc. (London),* **A113,** 606 (1927)
159. McGavack, J., U. S. Pat. 1,932,632 (Oct. 31, 1933)
160. Messmer, A. N., U. S. Pat. 1,373,518 (Apr. 5, 1921)

161. Morgan, W. L., *Ind. Eng. Chem.*, **23**, 505 (1931); see also Greene, O. N., *Modern Plastics*, 38, Jan. 1937
162. Mullady, J. B., U. S. Pat. 1,779,613 (Oct. 28, 1930)
163. Nabenhauer, F. P., *Ind. Eng. Chem.*, **22**, 54 (1930)
164. Nowak, O. H., U. S. Pat. 809,731 (Jan. 9, 1906)
165. Obst, W., *Kunstdunger und Leim*, **28**, 439 (1931)
166. Ohl, F., *Gelatine Leim Klebstoffe*, **4**, 164 (1936); **5**, 35 (1937)
167. Perry, T. D., *Modern Plastics*, (Dec., 1936); Perry and Breth, M. F., *Amer. Soc. Mech. Eng., Woodworking Industries Div.* (1937)
168. Perry, T. D., *Furniture Mfr.*, **53**, No. 8, 16 (1938)
169. Pierson, G. G., U. S. Pat. 1,964,960 (July 3, 1934)
170. Portheim, E. R. v., U. S. Pat. 241,897 (May 24, 1881); 243,178 (June 21, 1881)
171. Prestholdt, H. L., U. S. Pat. 1,506,201 (Aug. 26, 1924)
172. Prestholdt, H. L., U. S. Pat. 1,506,202 (Aug. 26, 1924)
173. Prestholdt, H. L., U. S. Pat. 1,506,203 (Aug. 26, 1924)
174. Prestholdt, H. L., U. S. Pat. 1,535,375 (Apr. 28, 1925)
175. Prestholdt, H. L., U. S. Pat. 1,604,307 (Oct. 26, 1926)
176. Prestholdt, H. L., U. S. Pat. 1,604,308
177. Prestholdt, H. L., U. S. Pat. 1,604,309
178. Prestholdt, H. L., U. S. Pat. 1,604,310
179. Prestholdt, H. L., U. S. Pat. 1,604,311
180. Prestholdt, H. L., U. S. Pat. 1,604,312
181. Prestholdt, H. L., U. S. Pat. 1,604,313
182. Prestholdt, H. L., U. S. Pat. 1,604,314
183. Prestholdt, H. L., U. S. Pat. 1,604,315
184. Prestholdt, H. L., U. S. Pat. 1,604,316
185. Prestholdt, H. L., U. S. Pat. 1,604,317 (Oct. 26, 1926)
186. Prestholdt, H. L., U. S. Pat. 1,701,763 (Feb. 12, 1929)
187. Rauppach, E. and Bergel, L., U. S. Pat. 500,428 (June 27, 1893)
188. Regnier, A. E., U. S. Pat. 1,506,081 (Aug. 26, 1924)
189. Renken, F., U. S. Pat. 729,220 (May 26, 1903)
190. Reuter, L. H., U. S. Pat. 1,167,434 (Jan. 11, 1916)
191. Robertson, J. H., British Pat. 303,332 (June 25, 1928); 306,351 (June 25, 1928)
192. Rolet, A., *Caoutchouc et gutta-percha*, **24**, 13,743, 13,784, 13,823 (1927); *Mat. grasses*, **19**, 7981, 8008, 8031 (1927)
193. Ross, J. H. and Ross, C. D., U. S. Pat. 183,024 (Oct. 10, 1876)
194. Sanford, P. G., U. S. Pat. 607,281 (July 12, 1898)
195. Sauer, E. and Hagenmüller, K., *Kolloid-Z.*, **83**, 210 (1938)
196. Scherer, R., U. S. Pat. 1,409,472 (Mar. 14, 1922)
197. Schmidt, A., U. S. Pat. 670,689 (1901)
198. Schroeder, W., U. S. Pat. 1,604,664 (Oct. 26, 1926)
199. Schultz, M. E., *Milchwirtschaft Forsch.*, **11**, 294 (1930)
200. Schwartz, F. F., British Pat. 405,381 (Feb. 8, 1934)
201. Serle, P. H. W., British Pat. 163,349 (Aug. 22, 1919)
202. Shaw, R. H., *J. Ind. Eng. Chem.*, **12**, 1168 (1920)
203. Shisler, G., U. S. Pat. 1,886,750
204. Slough, J. T., U. S. Pat. 709,651 (Sept. 23, 1902)
205. Sorensen, *Furniture Mfr.*, **46**, 21 (July, 1933); **47**, 23 (June, 1934)
206. Sponsler, O. L., Dunlap, M. E., and Henning, S. B., U. S. Pat. 1,336,262 (1920)
207. Statham, N., U. S. Pat. 1,209,221, 1,209,222 (Dec. 19, 1916)
208. Steffens, J. G. W., U. S. Pat. 169,053 (Oct. 19, 1875)
209. Stout, L. E. and Brew, W. B., *Plastics*, **15**, No. 7, 39 (1938)
210. Supp, F., U. S. Pat. 974,448 (Nov. 1, 1910)
211. Talman, J., U. S. Pat. 852,915 (May 7, 1907)

212. Thube, G. A. and Preaubert, L., U. S. Pat. 788,857 (May 2, 1905)
213. Truax, T. R., *Amer. Soc. Mech. Engrs., Wood Industries Div., Trans.,* **54,** No. 4, 1 (1932); *Wood Working Industries* (May, 1930); *Timberman* (May, 1930); *Amer. Soc. Mech. Engrs., Aeronautics Div., Trans.,* **61,** 1928; *Furniture Mfr. & Artisan* (May, 1924)
214. Truax, T. R., Browne, F. L., and Brouse, D., *Ind. Eng. Chem.,* **21,** 74 (1929)
215. Truax, T. R., *U. S. Dept. of Agric. Bulletin* 1500 (1929); *Technical Bulletin* 205 (1930)
216. Truax, T. R., *Veneers* (Oct. 1930)
217. Türmerleim-Werke Pfeiffer & Schwandner G.m.b.H., French Pat. 657,806 (May 27, 1929)
218. Vining, G., U. S. Pat. 223,459 (Jan. 13, 1880)
219. Wampler, R. W., U. S. Pat. 1,963,569 (June 19, 1934)
220. Wappes, H. and Klingenberg, H., U. S. Pat. 1,959,185 (May 15, 1934)
221. Watkins, G. B. and Harkins, W. D., *Ind. Eng. Chem.,* **25,** 1187 (1933)
222. Watkins, G. B. and Ryan, J. D., *Ind. Eng. Chem.,* **25,** 1192 (1933)
223. Wedger, W. H., U. S. Pat. 1,967,639 (July 24, 1934)
224. Weelands, W. A. and Williams, E., U. S. Pat. 1,015,365 (Jan. 23, 1912)
225. Wendt, G., U. S. Pat. 617,210 (Jan., 1899)
226. Whitehead, T. H., *Ind. Eng. Chem., Anal. Ed.,* **5,** 150 (1933)
227. Wilcken, H., German Pat. 591,550 (Jan. 23, 1934)
228. Wittkowsky, C., U. S. Pat. 537,096 (Apr. 9, 1895)
229. Zoller, H. F., *J. Gen. Physiol.,* **3,** 635 (1920)

Chapter 9

Casein in Paper Making

E. Sutermeister

The use of casein in the paper industry is confined very largely to the preparation of coated or enameled papers, though there are a few other lines in which it is, or has been, used to a slight extent.

Nature of Coated Paper

Coated paper, which is also termed glazed, enameled or art paper, was developed within a comparatively recent period to satisfy the demands of printers for a paper upon which fine half-tones could be reproduced with good results. Such paper is prepared by mixing mineral matters with a solution of an adhesive and applying this mixture in a thin, even layer to the surface of an ordinary sheet of paper. The function of the adhesive is to bind the mineral matter so firmly to the paper that it will not be removed or "picked" off during the printing operation, while the mineral matters form a surface which is receptive to ink. Such a coating covers the individual fibers on the surface of the paper and also fills any hollows between them, so that the paper, after calendering, has a smooth, even, and continuous surface, which permits the finest dots of the half-tone plates to reproduce perfectly. Coated papers are used for lithographic work, for magazine and other printing, and more especially for high-class advertising where the perfection required of the illustrations necessitates the use of half-tone plates with a large number of lines per inch.

Adhesives Used

There are at present three chief adhesives used in the coating of paper, namely starch, glue, and casein; but the amount of casein used far outweighs that of the other two combined.* There has been a strong prejudice against starch because many coaters have attempted to use it without sufficient knowledge of its properties or the effects of modifying it by various chemical treatments. This resulted in the preparation of much paper that was weakly coated and would not print without picking, and

* In Germany, inadequacy of German production of casein for the needs of the paper industry appears to be a cause of concern in the effort to make the nation self-sufficient, both glue and starch being considered inferior to casein.[10]

as a consequence starch in coating has been regarded as an evidence of inferiority. This is very unfortunate, for when properly treated and applied starch will give as strong and permanent a coating as either glue or casein, and moreover it is not subject to putrefactive decomposition with resulting bad odors. Coated papers in which starch is the adhesive seem to absorb the printing ink a little more than do those with glue or casein coating; and while this necessitates the use of a little more ink it is not considered a serious defect.

Glue was practically the only adhesive used for some time after the development of coated paper. At that time it was found to vary considerably, even in shipments that were supposed to be the same, and it frequently resulted in weak-coated paper and defective printing. Another trouble with glue was the objectionable odor which it often imparted to the finished paper. This was sometimes due to the spoiling of the glue solution in the coating plant and sometimes to the inferior quality of the materials used by the glue maker; but in either case the result was an unsatisfactory paper. Better controlled methods in the glue industry have gone far toward curing these troubles and a strong effort is being made to reëstablish glue in the esteem of the paper coater. Whether this can be accomplished to any extent will depend considerably upon the relative cost of glue and casein.

Casein as an adhesive has one inherent advantage over either glue or starch—the ease with which it is rendered waterproof. Papers in which it is used can easily be waterproofed to such an extent that when moistened and placed in contact they will not adhere, nor will the surface be injured. If need be, the coating can be made so resistant to water that the paper can be washed with a sponge without injury. This same effect can be produced in glue-coated papers, but only with difficulty and at considerable expense. Where starch is used there is no method known for making the coating waterproof. Apart from this advantage the chief reasons for preferring casein to glue are those of cost and the unwillingness of coaters to change their methods of working.

Attempts have been made at various times to prepare solutions containing both casein and starch, or casein and glue. Such solutions do not appear to be homogeneous, and, especially with starch, there is a decided tendency toward curdling, or separation, of one or the other of the materials. In such a case the adhesive strength of the mixture is not equal to the sum of the strengths of the two components. In some instances it was even found that the starch did not add anything to the strength of the casein but acted merely as so much inert material. For this reason mixtures of the two are seldom used, though rarely a paper is found in the coating of which both casein and starch are present.

Early Attempts to Use Casein

Because of the destruction of records by fire it has proved impossible to establish the year in which casein was first used commercially in this country in coating paper, but it was probably between 1890 and 1900. Before that it is claimed that curd, or pot cheese, had been used on a small scale in several plants with excellent success as far as color, odor and adhesive strength were concerned, but that its use had to be abandoned because of the impossibility of preventing the curd from spoiling, especially in hot weather.

After the trials of green curd the next attempts were made with curd that had been dried and ground to the form in which casein is now found on the market. It was customary for the casein manufacturers or dealers to mix this casein with the alkali necessary for its solution before delivering it to the consumer, and such large quantities of borax and soda ash were used for this purpose that it almost seemed that the amounts were governed by the fact that the alkalies were cheaper than the casein. This practice left the paper coater no choice in the selection of his solvents and obliged him to work with very alkaline solutions. The high alkalinity of the dry casein also appeared to cause deterioration if the material was stored for any length of time, and this introduced another source of trouble. Little real progress was made in using casein until this custom was abandoned and the material delivered unmixed with alkali.

When casein was first used for coating paper it was being made as a by-product in many small creameries, and because it was a by-product little care was given to its manufacture. There was also no uniformity in the methods employed, some allowing the milk to sour itself, some adding acid and some using the rennet process. Sometimes the kinds of casein were kept separate and sometimes they were mixed, so that it was not only general to find the lots working differently, but it was not at all uncommon to find several barrels out of a carload that could not be used at all and had to be returned to the shipper. Under such conditions it was very difficult to locate the source of any trouble, and the confusion was increased by the fact that the users had little knowledge of the way to handle casein and were more or less prejudiced against it. Doubtless many lots of casein were rejected that could be used today without difficulty, but it is equally true that much was used that would today be rejected because of its lack of uniformity and because of the higher quality of modern papers.

Conditions are now greatly improved, both because the preparation of the casein is better standardized and looked after and because the paper coater knows much more about handling it to the best advantage. It is seldom that a lot of casein now has to be rejected because of poor quality, or even that unsatisfactory samples are received.

Coating Operations

In the preparation of coated papers five chief operations are involved: dissolving the casein or other adhesive, mixing the solution with the mineral matters to be used in the coating, spreading the completed mixture on the surface of the paper, drying the moist paper, and smoothing or polishing the surface by calenders or other means. The first three of these are influenced to a greater or less extent by the kind and quality of the casein used. Knowing the degree and nature of these influences is a very great help to the paper coater in his attempts to produce a uniform and high-grade paper. The drying and calendering operations are common to all coated papers, whether casein, glue or starch is used, and as they are practically uninfluenced by the kind or quality of the adhesive they need not be discussed here.

Preparing Casein Solutions

The first step in preparing a casein solution is to mix the dry casein with water for a sufficient time to moisten each particle thoroughly before adding the alkalies. The time required depends on the temperature of the water as well as upon the fineness of grinding of the casein. Increasing the temperature of the water decreases the time necessary for its absorption by the casein; but the temperature must not be carried too high as this may cause the casein to soften and cohere in dense, sticky masses not easily penetrated by the alkalies and therefore very difficult to dissolve. This takes place if the casein is added to water at 85°C. (185°F.) or over, but if the casein is mixed with cold water and the temperature then raised to the same degree, no such formation of masses occurs. It is probable that caseins differ in their tendency to cohere in this way, but no definite information regarding this property is available.

The fineness of the casein is also of considerable importance. If it is too coarse an undesirably long soaking is required to permit the water to penetrate the coarsest pieces, while if it is too fine it is likely to lump when it is mixed with water. Such lumps contain dry casein inclosed within a coating of wet particles. The alkali acts on only the outer portion and when the coating mixture containing such lumps is finally strained, they break open and permit particles of undissolved casein to be distributed in the finished mixture. Such a mixture causes transparent spots in the paper when the particles of casein are crushed during calendering. It is only when nearly all the casein is finely divided that this trouble develops and a moderate amount of fine material, mixed with the coarse particles, does no harm. Screening tests on a South American casein that worked very satisfactorily, and

on a domestic casein that was considered to be too finely ground, gave the data of Table 26.

TABLE 26.—Size Distribution of Granules in a Satisfactory and an Unsatisfactory Casein

Size class of granules	South American casein	Domestic casein
	per cent	*per cent*
Retained on 20-mesh sieve	0.5	1.2
Passed by 20-, retained on 40-mesh sieve	32.5	16.2
Passed by 40-, retained on 60-mesh sieve	38.3	18.8
Passed by 60-mesh sieve	28.7	63.8

Addition of Alkalies

After the casein is thoroughly soaked the next step is to add the alkalies; this is best done by dissolving them in a small amount of water and pouring the solution into the cooking vessel containing the casein. Among the alkalies that have been proposed are sodium sulfide, silicate, aluminate, sulfite, phosphates, and carbonates, as well as borax, caustic soda and ammonia. Potassium salts would doubtless work equally well but they are never used because of the expense. Of the alkalies mentioned the first four are seldom used on a commercial scale and the bulk of the work falls on the other five. The selection of a solvent depends to some extent on the personal preference of the coating room foreman but to a still greater degree on the type of paper being made. With some satin whites (satin white is a pigment made by treating slaked lime with aluminum sulfate) borax is likely to cause serious thickening, whereas with others it can be used in large amounts without trouble. The amount that can be used with safety can be found only by trial, but it is desirable to use as much of it as possible in order to take advantage of its preservative properties. Ammonia is a vigorous and rapid solvent which has the advantage of being driven off during the drying of the paper, thus rendering the coating distinctly waterproof, as well as giving it a neutral or slightly acid reaction toward litmus. This makes it particularly valuable for certain papers that must be free from soluble alkali. Caustic soda is one of the cheapest alkalies to use because of its great solvent power; but if used in excess it causes the solution to darken, sets up decomposition of the casein and acts destructively on the brushes of the coating machines. For these reasons it is generally avoided in the preparation of the solutions, but it deserves more attention than it gets and is safe to use wherever good technical control is maintained.

From the success attending the use of slaked lime as a solvent in making casein glues it would appear that it might have possibilities in paper coating work. It has the disadvantage of reducing the time

during which the coating mixture retains its good working properties, and from the few experiments that have been made it seems to increase very greatly the foaming properties of the coating. Gould and Whittier[9] also found that the presence of calcium compounds tended to lower the adhesive strength of the casein; but as this was true whether the element was present in the casein ash or added as inorganic compounds, and as calcium compounds are always present when satin white or precipitated chalk is used, there is still some doubt whether the use of lime as a solvent would cause any additional weakening in many cases. It is believed that a study of this solvent might be well worth while.

In general it may be said of the alkaline solvents that the best one to use, or the best combination, depends so much on the quality of the paper being produced, on the desired consistency of the coating mixture and on the coating equipment employed that it is impossible to generalize with safety. A study of the relation of these factors would be well repaid in most coating plants.

From practical trials with a large number of caseins the pounds of solvent necessary for just complete solution of 100 pounds of casein was determined. The results are given in Table 27, together with the chemically equivalent weights of the different solvents (Discrepancies between the data of Table 27 and those of Table 20 in Chapter 8 are probably due to the fact that the solutions reported in Table 20, except that in sodium fluoride, were made at room temperature, without warming the mixture.)

TABLE 27.—Minimum Quantities of Different Alkalies Required to Dissolve 100 Pounds of Casein

Alkali	Minimum quantity required	
	Pounds	Pound equivalents
Sodium hydroxide, NaOH	2.35 to 3 10	0 059 to 0.078
Borax, $Na_2B_4O_7 \cdot 10\ H_2O$ . .	14 7	0 077
Ammonium hydroxide, NH_4OH	3.15	0 090
Sodium carbonate, Na_2CO_3	5.14	0 097
Trisodium phosphate, $Na_3PO_4 \cdot 12\ H_2O$	12.3	0.097
Sodium sulfite, $Na_2SO_3 \cdot 7\ H_2O$	14.0	0 111

In some cases these alkalies are used individually, but it is quite general to use a mixture of two or more that will give the desired properties to the coating mixture. These combinations of solvents have been made up from the results of practical trials and it has proved impossible to predict the effect of any given mixture from scientific considerations.

In preparing solutions for use in paper coating the chief consideration is to obtain a complete solution, and any amount of alkali that will give such a solution will develop the full adhesive strength of the casein.

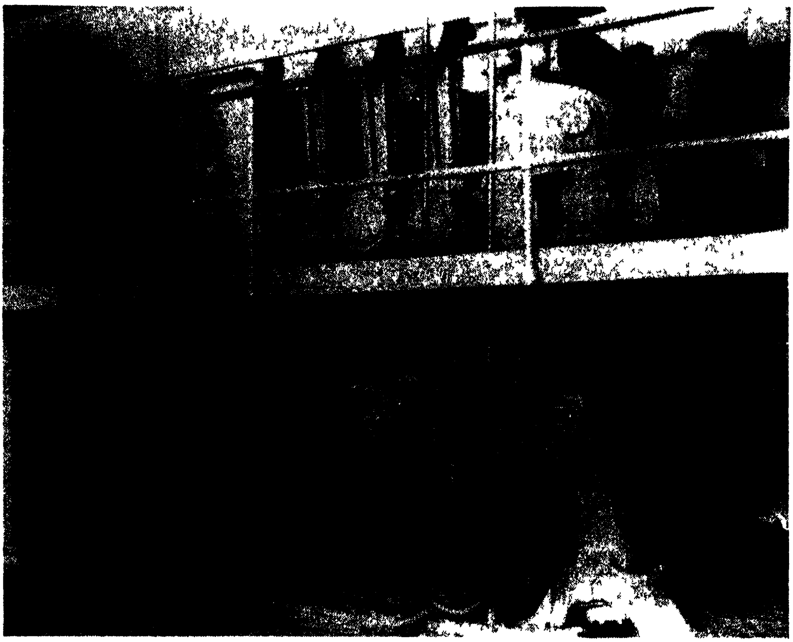
There may be an exception to this in the case of the phosphates, for Gould and Whittier[9] find that compounds containing phosphorus tend to lower the adhesive power of the casein. It has also been shown that, so long as the solution is complete, the temperature of cooking is without influence on the strength of the casein, unless the heating is so prolonged as to cause its decomposition. It was found, for instance, that a casein dissolved at 20°C. (70°F.) was neither stronger nor weaker than the same casein dissolved at a temperature of 86°C. (195°F.) for 15 minutes. Although such high temperatures are safe in laboratory work they should never be permitted where large quantities of solution are being prepared, because it requires so much time to cool a large body of solution that decomposition may take place to a considerable extent before a safe temperature is reached.

The amount of alkali used in preparing solutions for paper coating is generally considerably in excess of that required for a neutral or complete solution. In the case of most of the alkalies this excess does no harm, and it has been found necessary when much satin white is used. When ammonia is employed in considerable excess it darkens the solution to a marked degree and often tends to thicken it. Caustic soda in excess is very apt to cause decomposition of the casein into substances darker in color and of little adhesive strength. It is difficult to see how any workable solution could have been obtained by a Dutch formula of 1911, in which 15 parts of casein and 15 of caustic soda were dissolved in 70 parts of water. Such a solution must have had remarkably poor keeping qualities and probably was extremely destructive to the brushes.

The preparation of casein solutions is usually carried out in open tanks fitted with agitators. They are preferably of such shape that the solution may be drawn off rapidly and completely. The agitators should be of a type to give good mixing without splashing, for this will spatter the casein onto the sides of the tank where it will gradually collect and decompose, making it difficult to keep the apparatus clean and sweet. Formerly the heating was done by blowing steam directly into the contents of the cooker, but the more modern method is to cook in steam-jacketed tanks. This permits a better control of the density of the solution, since no water is added by the condensation of the steam, and it also makes it possible to cool the solution by passing cold water through the jacket. This has been found desirable because it gives lighter-colored solutions, and prevents decomposition of the casein when large amounts of alkali are used. Practical experience has shown that satisfactory solutions may be obtained by agitating at a temperature of 54° to 60°C. (130° to 140°F.) for an hour. As soon as it is certain that the casein is all dissolved the steam should be shut off and the solution cooled to 40°C. (100°F.) or below. This

is particularly necessary when the casein is to be mixed with satin white, for if the solution is too hot the mixture thickens so greatly that it cannot be applied to the paper.

When ammonia is used in conjunction with other solvents some authorities claim that it should not be added until the full temperature



Courtesy of the Consolidated Water Power and Paper Company

Figure 40.—A double coating machine for coating both sides of the paper in one operation.

of cooking has been reached and the steam shut off. This reduces the loss of ammonia through volatilization and permits it to act on the casein when the latter is thoroughly softened. In other plants this rule is not observed and the ammonia is added with the other solvents when steaming is started.

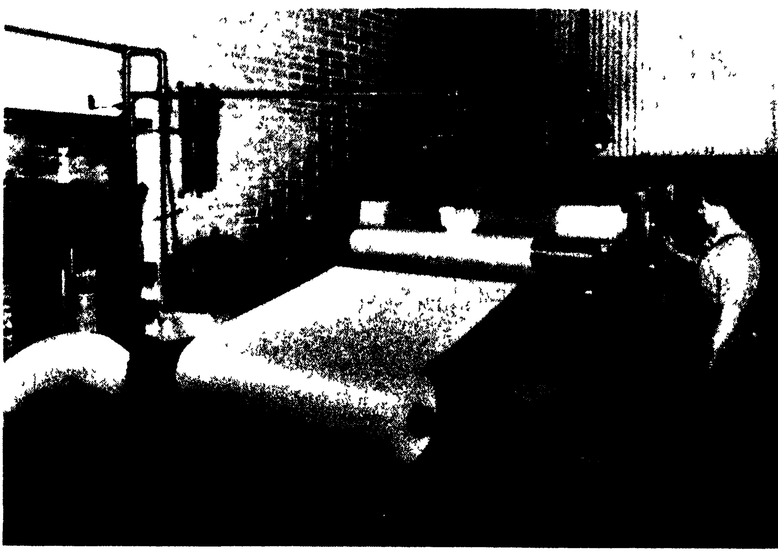
Typical Mill Procedures

In the preparation of casein solutions in the coating plant it is not always practicable to observe all the general rules just set down and in fact they would not all be subscribed to by every paper coater. It may therefore be of interest to describe three or four actual mill procedures. For the sake of convenience all the formulas in Table 28 are calculated to a basis of 100 pounds of casein.

TABLE 28.—Typical Formulas for Casein Solutions

Ingredient	Formula M	Formula N	Formula O	Formula P
Casein, pounds...............	100	100	100	100
Borax, pounds...............	5	4.5	3.2	5 5
Trisodium phosphate, pounds..	6.4	29.6	20 8	7.4
Soda ash, pounds.............		5.5		
Ammonia, 26°, gallons........	2	0.4	0.4	0.4

In the plant using formula **M** Clark[18] states that the casein is soaked in water one hour and the borax and trisodium phosphate, dissolved



Courtesy of the Champion Paper and Fibre Company

Figure 41.—Applying the coating to the paper. This machine coats both sides of the paper simultaneously.

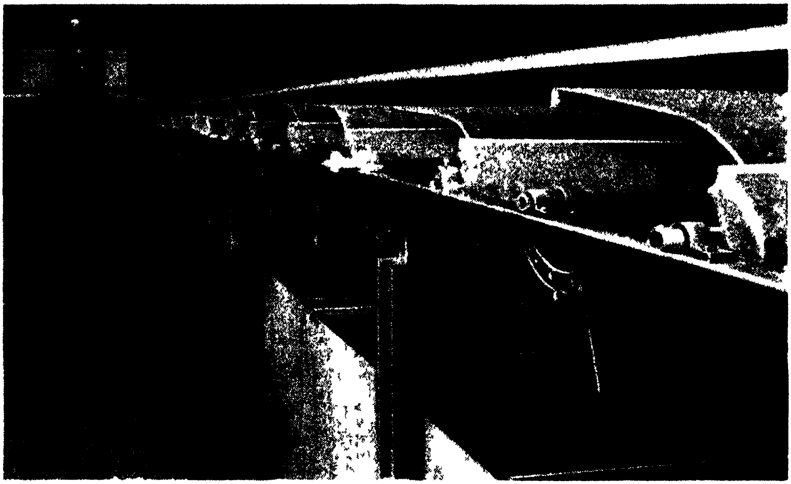
in water, are then added to it. The mixture is heated to 150°F. by passing steam through the jacket; steam is then shut off and the ammonia added. Cold water is then passed through the jacket until the temperature of the solution drops to 80 to 90°F. Agitation is continuous during cooking and cooling in order to avoid the formation of "skins" on the surface of the solution.

The plant using formula **N** adds the casein to the proper amount of water and as soon as it is thoroughly agitated and all casein is wet, the dry solvents are added. Steam at 90 pounds pressure is then blown directly into the charge until the temperature reaches 130°F., when it is shut off. During the heating period the ammonia is added

at the convenience of the workman and as soon as solution is complete the charge is cooled to 95 to 100°F. The last of the cooling is done without agitation to avoid whipping air into the thickened solution.

Formulas **N** and **O** have been used with good results when a considerable amount of satin white is present in the coating, while **P** works well with clay alone but is not satisfactory with satin white.

All casein contains more or less dirt and insoluble matter and in some cases it is desirable to clarify the solution before use. This can be done very satisfactorily by passing the hot solution through a cen-



Courtesy of the Champion Paper and Fibre Company

Figure 42.—The hot line. The paper is floated over steamheated grills from which a blast of warm air suspends the paper in the air while it is being dried.

trifugal separator in which the bowl is so constructed as to retain the dirt. This treatment makes it possible to use caseins that would otherwise be unfit for use in high-grade papers, but with most of the modern caseins, and except in cases where extreme cleanliness is desired, such clarification is quite unnecessary.

Preparing the Coating Mixture

After the casein solution is prepared the next step in the process is its incorporation with the mineral substances and other materials to produce the finished coating mixture.

The general method of preparing coating mixtures is to work up the mineral matters and water in an agitator which will thoroughly incorporate them, and then add the casein solution. The tinting materials and the foam reducers are added just before the mixture goes

to the coating machine. For reasons of economy it is desired to use as little casein as possible and still hold the coating on the paper strongly enough to resist the pull of the printing ink. This is also desirable because the more casein used the less gloss the paper will take on calendering, and the less readily will it absorb printing ink.

Mineral Substances Used

The mineral substances for the great bulk of coated paper consist of white pigments such as china clay, blanc fixe (precipitated barium



Courtesy of the Champion Paper and Fibre Company

Figure 43.—The festoon drier in which drying is completed The folds of paper are moved slowly along while suspended from the overhead track

sulfate), precipitated chalk (calcium carbonate), satin white, talc, titanium pigments, zinc sulfide pigments, etc. With these are mixed coloring materials of various kinds to give the tints or colors desired in the finished paper, and waxy or oily substances to improve the finish of the paper or reduce the foaming tendencies of the coating mixtures.

The minerals used vary with the kind of paper to be made; blanc fixe and precipitated chalk give dull or semi-dull finishes, clay a more glossy surface and satin white a still higher shine; titanium pigments and

zinc sulfide pigments impart opacity and whiteness. Apart from these differences the minerals also have a distinct effect on the proportion of casein necessary. This appears to depend partly on the fineness of the particles and partly on their chemical nature. Because of these variations it is difficult to give accurate comparisons of the percentages of casein required by different minerals but from laboratory tests, 100 parts of dry mineral matter appear to require approximately the following proportions of casein:

Blanc fixe	12	parts
Precipitated chalks	15 to 28	parts
Clays (English)	10.5 to 14	parts
Clays (American)	10 to 15	parts
Titanium dioxide	14	parts
Satin white	45 to 60	parts

The effect of degree of fineness has been proved by separating a clay into portions remaining in suspension in water for various lengths of time and testing the fractions. It was found that the finest part required several times as much casein as the coarsest. With four different clays, in which the portion remaining in suspension over two hours varied from 41.8 to 86.2 per cent, the casein required increased with increase in the fine fraction in the clay, the finest requiring about twice that needed for the coarsest. It is probable that the difference between clays is due to physical rather than chemical causes, but whatever the reason, it is quite possible that it is sometimes the reason for the casein being unjustly blamed for poor strength.

With satin white the fineness of particles may be responsible for the greater amount of casein required, over that necessary for clay, but here another factor enters, *viz.*, the chemical activity of the material. Satin white is formed by the interaction of slaked lime and aluminum sulfate and is probably calcium sulfoaluminate, $3CaO \cdot Al_2O_3 \cdot 3CaSO_4 \cdot 31H_2O$. It is of a strongly alkaline nature and it has been found necessary to hold its alkalinity, as expressed by direct titration with acid, within certain limits if trouble is to be avoided. If conditions are not right the coating mixture will thicken to such an extent that it cannot be spread. All the reasons for this change are not known, but it will take place if the alkalinity of the satin white drops too low, if borax is used for too large a proportion of the solvent, if a considerable excess of alkali is not used with the casein and if the solution is too hot when it is mixed with the satin white. Caseins differ considerably in the degree to which they thicken with satin white and it is quite probable that a careful study of the situation would enable a casein to be produced that would be much less likely to cause trouble than the present commercial products.

With precipitated chalks prepared by the causticizing reaction the

very great variation in casein required is partly due to fineness of particles and partly to residual impurities from the lime used. The relative effect of these two factors is not yet definitely known.

Coating the Paper

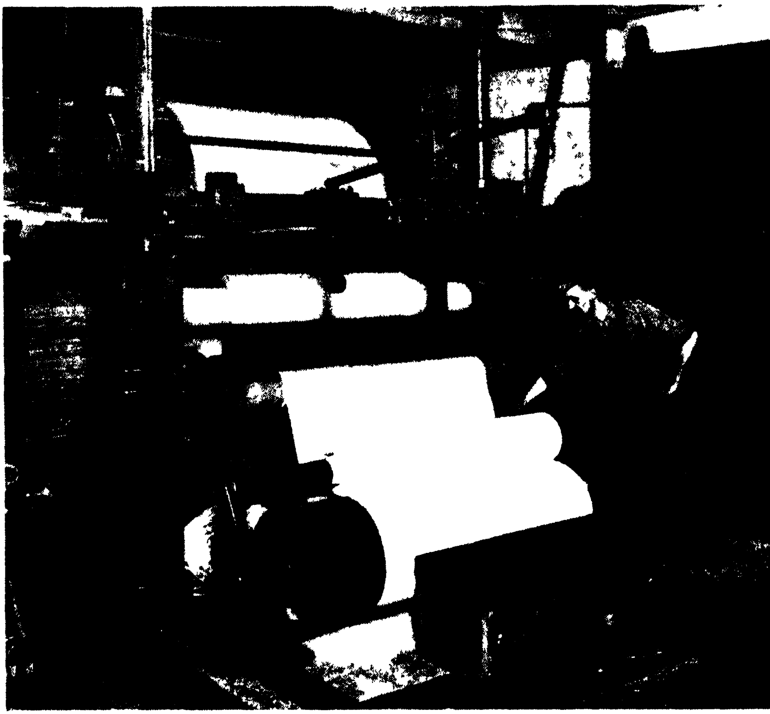
There are a number of types of coating machines, some of which coat one side of the paper at a time whereas others do both sides in one operation. In the latter the paper is drawn in a continuous web through a trough of the mixture, is next passed between squeeze rolls to remove all but the desired amount, and is then subjected to the action of brushes working back and forth across both surfaces. The brushes distribute the coating evenly and the paper is then dried over blasts of heated air and in festoons in a drying gallery. A description of various modern coating machines has recently been published.[5]

During these operations several troubles are apt to develop which are caused, in part at least, by the casein. If the latter is incompletely soluble the undissolved particles are likely to work up into the brushes until they collect in such quantities that the bristles can no longer hold them. They then drop off onto the paper and form lumps in the coating which are ruinous to the printing plates if they are not all removed during the sorting of the paper. If the fluidity of the coating mixture is not right it will not give good results. When it is too thin it may prove impossible to apply enough coating, and if it is too thick the surface of the paper may be full of brush marks. These are the marks caused by the bristles dragging through the coating; when its consistency is just right it is fluid enough so that it flows a little after it leaves the brushes and the marks disappear; but when it is too thick this flow is lacking and the tracks of the brushes show in the finished paper. The question of proper fluidity in the coating mixture comes down to the selection of the right solvents to go with the casein and minerals employed.

Foaming

A third trouble which is probably caused to a considerable extent by casein is that of foaming. If the coating mixture becomes very frothy it is likely to cause innumerable small pits in the coated paper, which may, at times, be bad enough to spoil its printing qualities. All the factors that cause the formation of foam are not known, but the fact that it varies with different caseins when all other conditions are constant throws considerable suspicion on the casein. Even in laboratory tests it can be proved that caseins vary widely in foam producing power. Recent studies by Whittier, Gould and Hall[20] have thrown some light on the problem. They found that foaming tendencies might be caused by too rapid agitation during the precipitation of the curd,

by the use of temperatures below 95°F. during precipitation, by insufficient washing of the curd, or by some unknown characteristic of the milk used. They offer the theory that the foaming tendency depends on the degree of aggregation of the casein molecule.



Courtesy of the Champion Paper and Fibre Company

Figure 44—The reel end of the coating machine where the completely dried coated paper is wound on reels before it is sent to the calender stacks for finishing

In the coating industry the foam is kept down to a safe point by the use of some material that causes the bubbles to break as soon as they are formed,[8] but even this is not always successful and the use of such materials sometimes introduces troubles that are as bad as froth. Whittier, Gould and Hall[20] state that foaming may be prevented by mixing even a small portion of a non-foaming casein with one of great foaming tendencies. It is believed that the makers of casein should give this subject much more study.

Defects in Casein

Casein as placed on the market is subject to several defects, most of which can be eliminated if proper care is taken during its prepara-

tion. It is only fair to say that they are much less frequent than formerly, indicating better coöperation on the part of producer and consumer.

Odor in casein cannot be entirely avoided as it has a characteristic odor when prepared by commercial methods. That this is due to impurities is indicated by the fact that pure casein, as prepared by laboratory methods, is nearly odorless. Unless casein is handled very carelessly during manufacture, thus permitting decomposition to set in, its odor is not bad enough to be objectionable to most persons; but if putrefaction begins, either during manufacture or during use in the coating plant the odor of the finished paper may be quite objectionable.

If casein is dried at too high a temperature, or for too long a time it may become discolored and difficultly soluble. Red-brown or "burned" particles in casein used to be a frequent cause of complaint, but of recent years they are seldom seen. It was generally the case that only part of the casein was thus discolored and the rest was of normal appearance, indicating a blending of burned and properly dried caseins by the manufacturer.

If casein is purchased in the form of coarse curd and ground at the point of use, trouble may be experienced if the drying has not been carried far enough. Sometimes the larger lumps contain cores of moist material that is of a rubbery consistency and resists the disintegrating action of the grinder. This is a very unusual form of trouble.

It is claimed that an excessive percentage of fat in casein will cause trouble in the coating by producing "eyes" or grease spots. There is no reliable information as to the proportion of fat permissible in a satisfactory casein and it probably varies with the type of work being done. Buttermilk casein contains more fat than that made from skim milk, and one instance is known in which a lot containing 12 per cent of fat was used without trouble. This was in the early days when the quality of paper was not equal to that made now, and there is some doubt about casein of that quality being used successfully at present.

A frequent cause for complaint used to be the presence of white flakes, which were sometimes visible in the dry casein, and would not go into solution when the casein was dissolved. These were often attributed to albumin, precipitated with the casein because of too high a temperature when treating the milk with acid. It was finally proved that they consisted of the hyphae of molds and that they were present because drying of the casein was so long delayed that the moist curd had a chance to mold. Proper handling of the curd entirely eliminates this trouble and it is unusual to find white flakes in modern caseins. This defect in casein is the cause of two troubles in coated paper; the white flakes cause little spots in the paper that do not print well, and they

sometimes work up into the brushes of the coating machines and collect until in sufficient quantity to fall off and make bad spots in the paper.

The foaming of coating mixtures has already been mentioned. This may or may not be due to defective casein, but it is strongly suspected that the impurities in the casein, or perhaps some of its degradation products, are largely responsible for its foaming. It is believed that this would be a fruitful field for research.

The deterioration of casein during storage is a fault that cannot be laid entirely to the casein, though some kinds keep better than others. If casein is stored in a warm place it is likely to become infested with larvae and after a while such casein will be found to have lost much of its strength and to contain considerable amounts of insoluble matter. In some cases caseins have been known to lose half their adhesive strength and to give such thin, watery solutions that the coating mixtures made from them were not of a workable consistency. The storage of caseins is discussed in Chapter 6.

Preservatives

When casein was first used in paper making much trouble was caused by casein spoiling in the coating plant. This resulted in very bad odors around the plant and in the paper itself, the latter feature being bad enough to cause much complaint from its users. As the putrefactive spoiling of casein also reduced its strength to a very marked extent, numerous lots of weak-coated paper were sent out, with disastrous results.

For a time the cause of this trouble was not fully realized and attempts were made to find a preservative or deodorizer that would not injure the quality of the paper. All sorts of disinfectants were tried and fair results were obtained with camphor and some of the essential oils. The use of borax was also found to be very beneficial in preventing the solutions from spoiling, and its use became very general. Gradually, however, the idea gained ground that more cleanliness in the coating plant and more care on the part of the casein maker would avoid this trouble, and in recent years there has been no cause for complaint regarding the putrefaction of casein in process. It is true that there is still a slight odor in paper coated with casein, but it is that due to the casein itself and is not caused in any way by spoilage. Occasionally an individual is found to whom this odor is objectionable but to the great majority of persons it is hardly noticeable.

If, in spite of these facts, the paper coater still desires to use preservatives as a measure of precaution, there are a number that are effective, among them being cresol, parachlorcresol, sodium orthophenyl phenate, beta naphthol, terpineol, and pine oil. The latter has the advantage of serving as a foam reducer as well as a preservative.

Waterproofing Agents

When casein is dissolved by sodium salts and used in a clay-coated paper the finished coating is not waterproof and may be removed easily by rubbing with a wet finger. If such papers become wet the sheets stick together so firmly that they cannot be separated without tearing and the book or other publication in which they were used is entirely ruined. This fault can be overcome by rendering the casein insoluble after it has been applied to the paper, and it was found that there were various means of accomplishing this. Treatment of the coated paper with a solution of such materials as ferric chloride, lead acetate, copper sulfate, chrome alum, zinc sulfate, etc., causes formation of the caseinates of the metals in question, and as these are insoluble the paper becomes waterproof to such an extent that it can be washed with a sponge. None of these materials can be added directly to the coating mixture before applying it to the paper, so their use involves an additional step in the manufacturing process, which is undesirable. Also many of the caseinates formed are colored, which prevents their use in white papers.

The most successful method of making the coating waterproof involves the use of formaldehyde. This is extensively employed in those grades of paper where the use of satin white is not general, as, for instance, in papers for covering boxes. It was found that if the formaldehyde were considerably diluted it could be added to the coating mixture without causing any change in its consistency, but that after this was applied to the paper and dried it gradually became waterproof. Too much formaldehyde or too strong a solution caused an immediate thickening of the coating mixture, and apparently part of the casein was rendered unavailable as an adhesive, for it was found necessary to use more than the ordinary amount to make the coating strong enough. About a pint of commercial formaldehyde for 100 pounds of casein caused the coating mixture to thicken only very slightly, but after standing one day it could not be thinned down by reheating. In practice rather less than this was found to develop sufficient waterproof qualities after the paper had stood for a short time.

Attempts have been made to use other materials, especially furfural and hexamethylenetetramine, as waterproofing agents. Furfural has not proved valuable for this work but hexamethylenetetramine has been used extensively and with considerable success. It is customary to prepare it when needed by mixing about equal volumes of formaldehyde and 26° ammonia; toward the end the ammonia is added very cautiously until the mixture has the odor of neither constituent. This mixture can be used in much larger proportion than formaldehyde without causing the coating mixture to thicken, and when subjected to the heat of the drying line it is broken down into its two constituents. The formal-

dehyde thus produced causes the waterproofing of the coating. Waterproofing with formaldehyde is not so necessary when satin white is used in the coating because the presence of the latter tends to cause waterproofness. This is probably due to the formation of insoluble calcium caseinate from the lime salts in the satin white. Doubtless the same result could be accomplished by the use of slaked lime as a solvent, if a method could be devised for employing it successfully. In some cases the use of ammonia as a solvent adds to the waterproof qualities of the coating, for the ammonia is driven off during the drying operation and the casein remains in an insoluble condition.

Other Uses of Casein

Addition of an alkaline solution of casein to the paper stock, followed by addition of alum, causes precipitation of casein on the fibers. This treatment is carried out in a beater, which, with respect to this process, acts merely in the capacity of a mixing device. The precipitated casein aids in cementing the fibers together and in preventing the fuzz that sometimes sticks up from the surface of paper. It does not assist in retaining clay or other filler, nor does it improve the resistance to penetration of the varnish that is sometimes applied in lithographic work. Papers treated with casein are firmer and have more snap and rattle than those not so treated, and it is probable that their strength is increased, though the evidence on this point is somewhat contradictory. There is a distinct tendency for casein in the beaters to cause foaming, and in some cases this has proved to be rather serious.

In the past considerable quantities of casein have been used in the beaters in the production of the so-called "onion-skin" grade of writing paper. There is no question about the good results that it gives in this paper, but the benefit derived is not proportionate to the cost of the casein, and its use has been entirely abandoned in favor of cheaper materials. So far as known this is true of its use in the beater for other grades and it is safe to say that the amount consumed in such ways is extremely small.

Casein has been proposed as an aid in the rosin-sizing process which imparts to paper the property of resisting the penetration of writing ink or other aqueous fluids. Here again contradictory results have been obtained. In one test where rosin and casein were made into a size together, 10 per cent of casein on the weight of the rosin being used, the sizing was no better than when no casein was employed. If casein is added to the beater to act as a dispersing agent and cause precipitation of rosin in colloidal dimensions it appears to improve the sizing very distinctly. One per cent on the weight of the materials in the beater increased the sizing of the sheet to 300 per cent of its value when no casein was present, while 0.5 per cent of casein increased it to 160 per

cent of its original value. These results are based on tests made by floating pieces of paper on a bath of writing ink and noting the time required for the ink to show on the upper surface. As strongly sized paper can be made more cheaply by other means, casein is seldom used for this purpose.

Some years ago attempts were made to use casein as a surface sizing agent for writing papers in place of glue. For this work the paper is run through a dilute solution of glue and then dried in any suitable way. When casein was used in alkaline solution, and properly diluted, it would give fairly good snap and ink resistance at first; but as the web continued to run through the bath of casein solution the acidity of the paper, due to the presence of alum, gradually neutralized the alkali and the casein began to precipitate in small flakes that spotted and ruined the paper. Continual addition of alkali would doubtless have overcome this difficulty, but the writing paper mills never favored the process sufficiently to justify further trials.

According to a report from the Department of the Interior at Ottawa,[13] casein can replace fish glue in making strong and durable waterproof and fire-proof asbestos paper, board, etc., and also in the preparation of high-pressure steam gaskets and the facing for brake linings. It is probably not very extensively used for such purposes, for it has been ascertained from three of the largest makers of asbestos products that they do not use it in any of their work. (See Chapter 13).

Casein has been used to some extent for various purposes in the wall-paper industry. It served as a finishing coat on certain heavy goods colored and embossed in imitation of leather; and in the papers known as "varnish tiles" it was used as a size coat to support the varnish. For the latter purpose its increasing cost made it too expensive and it was replaced by a vegetable product. It has been used in large quantities as the protective coating on washable wall papers, as is pointed out in Chapter 10. The solutions, or casein varnishes, used for this purpose must be neutral or nearly neutral in reaction to prevent detrimental effects on the dyes used for printing the papers.

In the manufacture of playing cards casein is used to some extent for the same purposes and in the same way as in the coating of paper. It is also used in conjunction with shellac dissolved with alkali as a top varnish for playing cards, for the top coat of two-coat waterproof papers, and for sizing in hard-sized sheets. It can also be used, with an after-treatment with formaldehyde, for oil- and waterproofing art papers, cartridge cases, buckets, and bags, etc. Though it may be used in small quantities in the production of these, and other paper converting specialties, the amount so consumed is insignificant compared with that employed in the coating of paper, and a description of the latter industry is therefore fairly representative of the use of casein in paper making.

Kinds of Casein Used

As explained in Chapter 4, casein is prepared by treating milk with rennet, with acid, or by allowing it to sour itself. Each of these processes imparts certain characteristics to the casein and with any single process the method of carrying it out and the severity of the treatment are also influential. It is not to be wondered at, therefore, that the product varies more or less and that this was particularly true in the early days when there was almost no uniformity in the methods of production.

Of the various kinds of casein made, that produced by treatment with rennet is not used in paper coating because it is not sufficiently soluble in alkaline solutions. This was a frequent source of trouble when casein was first introduced, for rennet casein was then often mixed with that made by the other processes and the insoluble part made it impossible to use the rest.

Naturally soured, or self-soured casein was formerly the first choice of the majority of paper coaters. Argentine casein, which is made in this way, has always had an excellent reputation for uniformity, good working qualities, and freedom from foam when in coating mixtures.

Casein precipitated by adding acid to the skim milk varies considerably in its properties and it was formerly thought that this was due to the kind of acid used. This opinion was probably based on the observation that muriatic casein, or that precipitated by hydrochloric acid, gave much thicker solutions and coating mixtures than that precipitated by sulfuric acid, and that both were of higher viscosity than those made from self-soured casein. A careful series of tests was carried out in 1922[17] on caseins especially prepared by precipitation with different acids, sulfuric, phosphoric, acetic, lactic, hydrochloric, and mixtures of the last three with hydrochloric; and as far as viscosity of solutions or coating mixtures was concerned comparatively slight differences were observable among them. The mixtures made with all of them were so thick that they could not be tested with the viscometer available, while that made from a self-soured casein was thin and fluid at the same temperature and gave a low reading for viscosity.

The ash in all these caseins ranged from 5.50 to 6.57 per cent whereas that in the self-soured sample was only 2.98 per cent. Tests were therefore made to see if treatment of the commercial caseins with hydrochloric acid would reduce the ash and, at the same time, the viscosity of the coating mixtures made from the treated casein. It was demonstrated that the ash could be reduced to 2.0 per cent, or even less in some cases, and that this change caused the coating mixtures to become nearly as fluid as that made from self-soured casein. Sulfuric acid was not nearly so effective as hydrochloric in reducing the viscosity and the percentage of acid used had a great influence on the results; the more acid em-

ployed, the lower the viscosity. The acid treatment causes a loss in weight of 11 to 15 per cent of the original casein, but the strength of the treated casein is enough greater to slightly more than counteract the loss in weight.

Since these remarks were first written great improvements have been made in manufacturing methods and the supposed differences between sulfuric, muriatic, and self-soured caseins have been shown to be non-existent in the products of the most up-to-date plants. Muriatic casein of the highest quality and possessing all the good qualities formerly attributed only to self-soured casein is now made in many plants, and the designation of caseins by the name of the acid used for precipitation no longer has any meaning in the trade.

Buttermilk casein has been used to some extent in paper coating with very variable results. Some lots would be of light color, and good solubility and strength, while others were reddish brown and contained so much insoluble matter that they could not be used in the ordinary way. Even under those conditions the soluble part was of good strength and if the solution were clarified by centrifugal action it could be used with good results. It is quite certain that, if properly made, buttermilk casein can be used to good advantage in coating paper.

A study of the viscosities of casein solutions and coating mixtures was made by the writer in 1919[16] in which it was shown that the viscosity of a casein solution depended on the kind of casein and also on the kind and amount of solvent used. The same thing was true of coating mixtures containing clay and satin white; but a casein giving a thin solution did not necessarily give a thin coating mixture, and it did not prove possible to grade a casein as to its value in coating paper by viscosity tests of its solutions alone. The work already done has developed a valuable means of testing caseins for use in certain grades of paper but it should be carried very much further in order to develop its maximum usefulness. It is highly probable that an intensive study along this line would develop basic facts that would be extremely useful to the casein maker as well as the paper coater, and that they would lead to a much more intelligent use of casein.[2, 4, 7, 15, 19]

The list of references includes a few general papers on the use of casein in the paper industry.[1, 3, 6, 11, 12, 14]

References

1. Anonymous, *Paper Trade J.*, **96**, No. 25, 34 (1933)
2. Anonymous, *La papeterie*, **58**, No. 20, 921 (1936)
3. Bryson, H. C., *Synthetic and Applied Finishes (London)*, **4**, 96 (1933)
4. Cobb, R. M. and Lowe, D. V., *J. of Rheology*, **1**, 158 (1930)
5. de Guehery, P. and Milham, E. G., *Paper Trade J.*, **106**, No. 26, 76 (1938)
6. Derby, H. L., *Paper Ind.*, **1**, 629 (1919)
7. Eldridge, E. F., *Mich. Eng. Expt. Sta. Bull.*, No. 42, 3 (1932)

8. Esch, W., *Gelatine, Leim, Klebstoffe,* **2,** 51 (1934)
9. Gould, S. P., and Whittier, E. O., *Ind. Eng. Chem.,* **24,** 791 (1932)
10. Hoyer, F., *Gelatine, Leim, Klebstoffe,* **3,** 174 (1935)
11. Matagrin, A., *La papeterie,* **47,** 1097 (1925) ; **48,** 121, 170 (1926)
12. Miller, R., *La papeterie,* **43,** 157 (1921)
13. Natural Resources Intelligence Service, Dept. of the Interior, Ottawa, Canada, "Casein and its Industrial Applications with Special Reference to Canada"
14. Regnier, A. E. and Dreshfield, A. C., *Paper Trade J.,* **89,** No. 24, 66 (1929)
15. Shaw, M. B., Bicking, G. W., and O'Leary, M. J., *National Bureau of Standards, Research Paper,* No. 254 (Dec. 1930)
16. Sutermeister, E., *Paper,* **24,** 217 (1919)
17. Sutermeister, E., *Paper Ind.,* **5,** 1224 (1922)
18. Technical Assoc. of the Pulp and Paper Industry, "Manufacture of Pulp and Paper," **5,** sec. 4, p. 5, New York, McGraw-Hill Publishing Co., 1925
19. Whittier, E. O., Gould, S. P., Bell, R. W., Shaw, M. B., and Bicking, G. W., *Ind. Eng. Chem.,* **25,** 904 (1933)
20. Whittier, E. O., Gould, S. P., and Hall, S. A., *Ind. Eng. Chem.,* **25,** 1213 (1933)

Chapter 10

Casein Paints

ALBIN H. WARTH AND FREDERICK L. BROWNE

Although casein paints of the quality now available are a comparatively recent development, the use of casein as a vehicle (binder) for paint is an exceedingly old art. Ancient Hebrew texts mention the decorator or painter. They admonish the housewife to store curd and the husband to go forth into the hills to bring back the color earths for the autumn visit of the decorator, when the house was to be painted for the Succoth. This system of painting has continued down to modern times in the villages of many parts of middle Europe. In rural Hungary, for example, the housewife prepares the clabber from skim milk while the painter brings freshly burned lime and earth colors to mix the paint on the job. In these countries the houses are usually of masonry with roofs of thatch, slate, or tile and it is principally the interior walls, commonly of plaster, that are painted.

During Colonial days and the early history of the United States whitewash played a more important part in painting, both exterior and interior, than oil paints, which at first were distinctly luxury products. Presumably whitewash, which is essentially slaked lime and water, was quite frequently mixed with the curds from skim milk. Whitewash remained one of the popular forms of paint down to the close of the nineteenth century, when its usefulness was immortalized by Mark Twain in the fence-painting episode in *Tom Sawyer*. It still enjoys wide use but the most serviceable formulas call for substantial proportions of casein.[149] Whitewash and the ancient home-mixed casein paints were the forerunners of the manufactured calcimines and casein paints.[174]

Factory-made casein paints in powder form, ready to be mixed with water for application, have been sold in the United States since the eighteen nineties.[1, 58a, 61, 86a, 97a] The casein paint business is therefore nearly as old as the "ready-mixed" oil paint business[92, p. 23] and fully as old as the manufacture of "flat wall" oil paints. Until about 1928, however, casein paints were negligible in amount in comparison with flat wall paints or with glue-bound calcimines. A striking increase in production of paints containing casein in recent years followed closely upon the development of greatly improved paints that could be sold in the form of soft pastes in which the casein is already in solution, requiring

only a simple dilution with water for application. At the same time the powder paints were improved in ways described farther on and have likewise gained a wider market.

New developments took place both in the United States and in Europe but along somewhat different lines. The American paste paints are predominantly casein-bound paints, though sometimes the casein is supplemented with other binding agents. Many of the new European paints to be thinned with water are emulsion paints in which the liquid portion of the paint is an emulsion of the oil-in-water type and the casein is the emulsifying agent to prevent separation of the liquid phases;[19, 57, 94, 124, 154, 173, 203, 213] but powder and paste casein paints essentially the same as the American products are also used widely.[13, 18, 30, 56, 64, 86, 89, 94, 101, 125, 138, 139, 163, 223]

Some General Principles of Paint Formulation

According to the American Society for Testing Materials,[4, p. 827] paint is "a mixture of pigment with vehicle, intended to be spread in thin coats for decoration or protection, or both," pigment is "the fine solid particles used in the preparation of paint, and substantially insoluble in the vehicle," and vehicle is "the liquid portion of paint." The definition omits some very essential requirements, namely, that the thin coats when spread must solidify reasonably promptly and adhere to the underlying surface, that the coating must then hide the underlying surface, and that it must provide color and a degree of gloss or absence of gloss ("flatness"), as desired. Enamel is more satisfactorily defined by the Society: "A special kind of paint which flows out to a smooth coat when applied and dries to a smooth, glossy, relatively hard, permanent solid when exposed in a thin film to the air. An enamel always contains pigment and has considerable hiding power and color. Some enamels dry to a flat or eggshell finish instead of a gloss finish." As to the distinction between paint and enamel the reader is left to his own resources. It may be suggested, however, that enamel usually forms a coating richer in vehicle than does paint, that the vehicle is harder, and that the coating levels out without such irregularities as the brush marks characteristic of paint.

The Nature of Pigments and Vehicles

Much the same palette of pigments, subject to certain limitations, is available for use in all of the important kinds of paint. It is convenient to divide the pigments into three groups:[36]

1. The opaque white pigments, which have an index of refraction greater than 1.9 and therefore make white paints when dispersed in vehicles.

2. The colored pigments, which absorb light selectively and usually make opaque paints.

3. The pigments of low opacity, which have an index of refraction usually between 1.5 and 1.7, do not make opaque paints when dispersed in such vehicles as linseed oil, and are commonly used chiefly for pigment bulk at minimum cost. For reasons discussed farther on, however, the pigments of low opacity provide a substantial degree of opacity in casein paints and calcimines.

For the vehicle of paint many substances are suitable theoretically and a number of them are used in practice. The fundamental requirements are that they be liquids or obtainable as emulsions, solutions, or sols of appropriate viscosity in which the pigments are substantially insoluble and capable of dispersion, and that, under controllable conditions, they solidify without separation of phases, without coagulation of the dispersed pigments, and with adherence to the underlying surface. The glasses and the colloids that form jellies obviously meet the requirements; both are used practically, the glasses in vitreous enamels, nonaqueous colloids in oil paints and lacquer enamels, and aqueous colloids in casein paints, emulsion paints, and calcimines. When aqueous colloids are used their jellies must be irreversible, that is, insoluble in water, if the paint is to be washable or suitable for exterior exposure.[172, 193] As the last two sentences suggest, paints are classified broadly according to the nature of the vehicle because that determines the basic properties of the paint, although further classification according to the nature of the pigment has been found necessary in the case of linseed oil paints.[36]

As a rule the vehicle of paint consists of a volatile and a nonvolatile part. In the solidified coating of paint, of course, only the nonvolatile part remains as the vehicle, but the volatile part is required for one or more of the following purposes: (1) to reduce the viscosity of an inconveniently viscous vehicle such as varnish; (2) to dissolve an otherwise solid vehicle such as casein, animal glue, or nitrocellulose; (3) to provide sufficient liquid to disperse the pigment in paints in which the volume of nonvolatile vehicle is insufficient for the purpose; (4) to make fine adjustments in the consistency of the paint; and (5) to act as a convenient solvent for introducing metallic driers in oil paints.

The volatile part of the vehicle is called the volatile thinner, though the American Society for Testing Materials excludes water in its definition of volatile thinner.[4, p. 828] The volatile thinner in oil paints and enamels is commonly mineral spirits or turpentine and in lacquers a mixture of organic esters, alcohols, ketones, and hydrocarbons. Because painters add water to calcimines, emulsion paints, and casein paints they are often called water paints or, where the water need not be hot, cold-water paints. Such terms are objectionable.[25] By the same logic flat wall paints of the oil paint class would be called mineral-spirits paints.

Moreover the use of water is not confined to the water paints, for it is not uncommonly an ingredient of nearly all types of oil paints, sometimes in very substantial amount. In the water paints, of course, water is used of necessity, whereas in oil paints it is a sophistication. The historically accurate collective for the water paints is distemper,[25] which is the term still used in England,[19] but in the United States it has become restricted to artists' water colors and fresco colors.

Proportions of Pigment and Vehicle

The proportions of pigment, nonvolatile vehicle, and volatile thinner in paints vary exceedingly widely and with fundamentally important con-

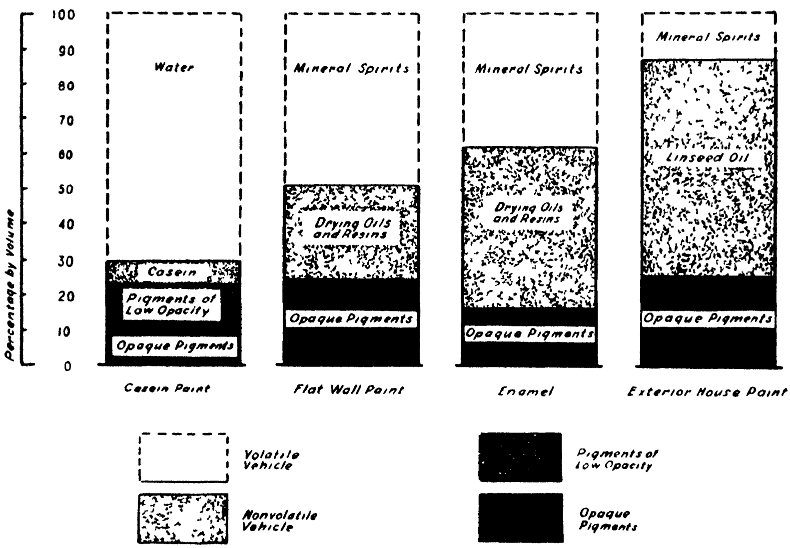


Figure 45.—Proportions by volume of pigments, nonvolatile vehicle, and volatile vehicle in casein paint, flat wall paint, enamel, and exterior house paint.

sequences.[208] Figure 45 shows diagrammatically the relative proportions by volume of pigment, nonvolatile and volatile vehicle in casein paint and in three kinds of oil paints, namely, flat wall paint, interior enamel, and exterior house paint, when each is mixed ready for the painter to apply. The data are based on the current specifications of the Federal Specifications Executive Committee for the kinds of paint in question[68, 69, 70, 71] except that for enamel an older specification is used because the current one does not define composition closely enough for the purpose. Commercial products, of course, vary widely in details of composition but they necessarily conform to the general trends revealed.

The casein paint is assumed to have the following composition:

	Pounds	Gallons	Percentage by volume	
Lithopone (28% zinc sulfide).	53 5	1.49	10.5	
Total opaque pigment....				10.5
Whiting (calcium carbonate)	23.1	1.03	7.2	
Calcium hydroxide...	12 5	.72	5.0	
Trisodium phosphate.	.9	.04	.3	
Total pigments.				23.0
Casein..... ..	10.0	.95	6 7	
Total nonvolatile				29 8
Water...	83 3	10.00	70 3	
Total paint	183.3	14 23		100 0

The ingredients are listed in the form in which the paint manufacturer uses them. When dissolved in water the trisodium phosphate forms a calcium phosphate with part of the calcium hydroxide; and the sodium hydroxide released, together with another part of the calcium hydroxide, reacts with the casein. In the dry paint coating the vehicle is therefore a mixture of sodium and calcium caseinates, not isoelectric casein, and calcium phosphate and excess calcium hydroxide are present as very finely divided, possibly colloidally dispersed pigment. As pigments they undoubtedly should be classified among the pigments of low opacity together with whiting.

The flat wall paint in Figure 45 is assumed to have the following composition:

	Pounds	Gallons	Percentage by volume	
Lithopone (28% zinc sulfide)	62 0	1 73	24 2	
Total opaque pigment....				24 2
Drying oils, resins, and driers.	15 2	1 90	26.5	
Total nonvolatile				50.7
Mineral spirits ..	22 8	3 54	49.3	
Total paint .	100.0	7.17		100.0

The interior enamel is assumed to have the following composition:

	Pounds	Gallons	Percentage by volume	
Lithopone (28% zinc sulfide)	37.5	1.05	12.8	
Zinc oxide	12 5	.27	3.3	
Total opaque pigment..				16.1
Drying oils, resins, and driers	30.0	3.75	45.9	
Total nonvolatile...				62.0
Mineral spirits...	20.0	3.10	38.0	
Total paint..	100.0	8.17		100.0

The exterior house paint is assumed to have the following composition:

	Pounds	Gallons	Percentage by volume	
Basic carbonate white lead	39.4	.69	12.1	
Zinc oxide	17.0	.36	6.3	
Titanium dioxide	4.8	.15	2.5	
Diluent allowance*			(6.5)	
Total opaque pigment				27.4
Magnesium silicate	6.8	.29	5.0	
Total pigments				25.9
Linseed oil and driers	27.2	3.51	61.1	
Total nonvolatile				87.0
Mineral spirits	4.8	.74	13.0	
Total paint	100.0	5.74		100.0

* Titanium dioxide is an extremely opaque pigment that is often used in the form of the composite titanium-barium pigment (25% TiO_2, 75% $BaSO_4$ by weight), which contains 2.6 parts of barium sulfate to 1 part of titanium dioxide by volume and yet is fully as opaque as white lead or zinc oxide. For that reason when pure titanium dioxide is used an allowance of 2.6 times its volume is added to the opaque pigments for more reasonable comparisons among different paints, but this diluent allowance is not added to the total pigments.[38]

In Figure 45 it is clear that, in exterior house paint and in interior enamel, the nonvolatile vehicle amounts to two or three times the volume of the total pigment. In flat wall paint the volumes of pigment and nonvolatile vehicle are approximately equal, but in casein paint the nonvolatile vehicle may be little more than one-fourth the volume of the total pigment.

Effect of Volume Relations on Structure of Coatings

The volume relations of pigment and nonvolatile vehicle profoundly affect the physical properties of the paint, particularly the optical properties of the resulting coating. In the liquid paint there must be enough total vehicle to disperse the pigment with ample room for the dispersed particles to slip by one another so that the paint will be fluid enough for proper application.[29 (p. 156), 32] It is also necessary that the viscosity of the vehicle be adjusted within reasonable limits. For that reason nonvolatile vehicles that are very viscous, such as the varnishes used in enamels, require a much higher proportion of volatile thinner than the vehicles that are very fluid, such as the unbodied (not heat-treated) drying oils used in good house paints. After application the volatile vehicle evaporates, diminishes the volume of vehicle, and therefore tends to make the coating shrink in thickness and to pack the particles of pigment closer together. In house paint and enamel, with enough nonvolatile vehicle to keep the pigment dispersed and embedded, the shrinkage in thickness is roughly proportional to the volume of volatile thinner lost; but in flat wall paints and casein paints, with insufficient nonvolatile vehicle for the dispersion of the pigment, the particles of pigment cannot

be packed together close enough to permit as much shrinkage as there is loss in volume from evaporation of volatile thinner. Instead, shrinkage

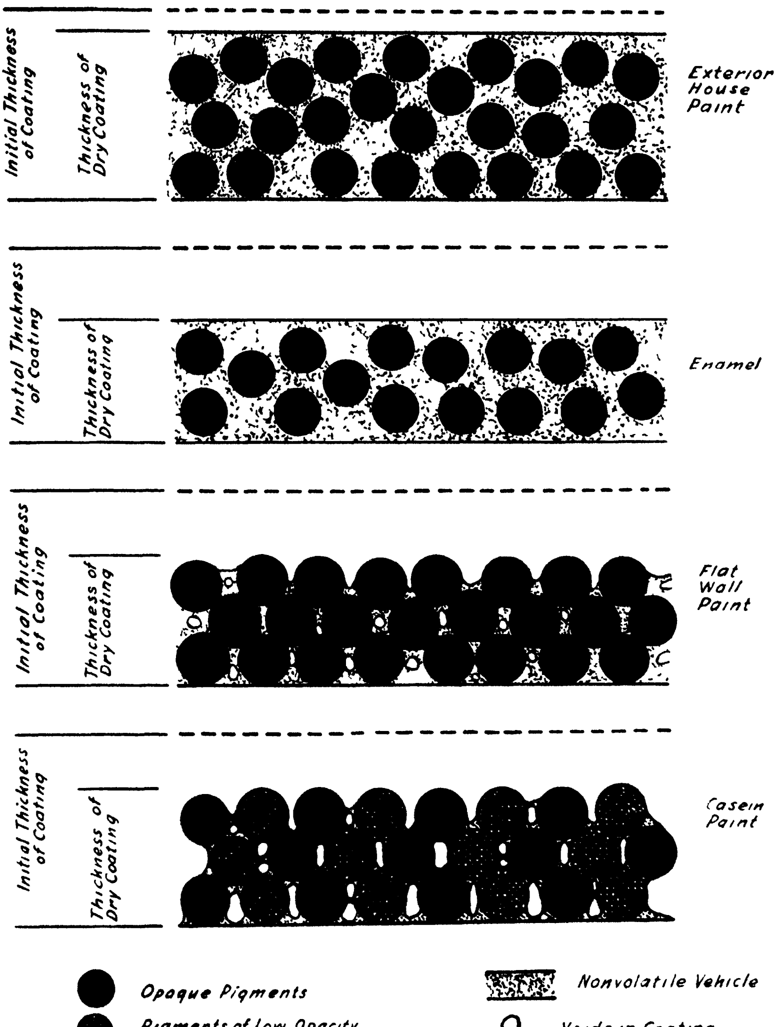


Figure 46.—Schematic representation of the shrinkage during drying, the distribution of pigments and nonvolatile vehicle, and the degree of porosity of casein paint, flat wall paint, enamel, and exterior house paint.

of the coating stops at a point determined by the pattern of packing characteristic of the particular pigment used[29 (p 157), 217] while the vehicle continues to recede within the voids between particles of pigment. When

dry the coating consists of a mass of pigment particles within which the interstices are filled in part by nonvolatile vehicle and in part by air.[81, 118]

Pfund[159] measured the thickness of coatings of different kinds of paint when freshly applied and again after drying and reported shrinkage in thickness amounting to 12 per cent for a linseed oil paint, 46 per cent for an oleoresinous enamel, 36 per cent for a flat wall paint, and 30 per cent for a cold-water paint. Accepting these data as typical, the structure of the final coatings of the four paints of Figure 45 may be represented schematically by Figure 46. Although the representation of particles of pigments as small "cannon balls" has been criticized[117] it is still a tempting boon to the writer who must be his own artist and takes comfort in the reflection that John Dalton's cannon ball molecules long served a useful purpose. A gross disproportion between the size of pigment particles in Figure 46 and the thickness of the coating will be forgiven by reason of obvious mechanical limitations. Of course particles of pigment in fact are rarely spherical in shape, uniform in size, or dispersed entirely without flocculation in the form of aggregates. Nevertheless Figure 46 aids in visualizing essential differences between the coatings formed by the four types of paint.

When the paint contains more than enough nonvolatile vehicle to fill the voids after the pigments have assumed their packing pattern, the coating consists of a solid matrix of hardened vehicle in which the pigment is completely embedded and the surface of the coating is the optically smooth surface of a jelly. Such a surface reflects much of the light that falls on it specularly, and is therefore glossy. Where the paint contains insufficient nonvolatile vehicle to fill the voids in the packed pigment, as in flat wall paint and casein paint, the coating is porous to a degree measurable by the insufficiency of the nonvolatile vehicle, and its surface is optically rough. Such a surface reflects light diffusely and therefore lacks gloss. The porous and nonporous coatings differ also in permeability to moisture, which determines their value as protective coatings. Porous coatings permit moisture to pass through readily and therefore have little protective value. Nonporous coatings transmit moisture only to the extent that it dissolves in the gelatinous matrix, diffuses to the opposite surface, and there escapes by evaporation or transfer to a hygroscopic substance such as wood. The resistance to moisture movement through a nonporous coating is greater the thicker the coating, the higher its concentration of pigment, and the lower the solubility of water in the matrix.[35, 63] Casein paint, therefore, has little value as a protective coating.

Optical Consequences of the Structure of Coatings

The porous and nonporous coatings differ not only in gloss but in other optical characteristics as well. The most important of these is the

amount and kind of pigment required to produce a given degree of opacity.[28a, 79 (p. 11), 88] Opacity arises from the reflection of light at the interfaces between vehicle and air, pigment and vehicle, and, in porous paints, pigments and air. Each interface encountered by a ray of light reflects part of the light, absorbs a small part (a large part if the pigment or vehicle is colored), and transmits the rest.[118] A large number of such reflections is required to reflect substantially all of an incident ray of light so that the light penetrates an appreciable distance into the coating before enough of it is turned back to hide the underlying surface. The lower the opacity of the coating the thicker it must be to hide what is beneath. The degree of opacity of the coating depends upon the number of interfaces encountered by a ray of light in traversing a unit thickness of the coating and the percentage of the light reflected at each interface.

In nonporous coatings the number of interfaces per unit thickness increases with the amount of pigment and with the fineness of subdivision of the pigment. If the particles become smaller than a wave-length of light, however, they merely scatter light instead of reflecting it and are therefore less effective from the point of view of opacity. Among zinc sulfide and zinc oxide pigments, for example, both the coarsest and the finest commercial grades are less opaque than the grades of intermediate particle size commonly used for paints.[218]

The percentage of light reflected at each interface increases with the difference in optical density on the two sides of the interface, which is conveniently expressed in terms of refractive index. The refractive index of some of the materials used in paints is as follows:

Air	1 000
Water	1 333
Nonvolatile vehicles	
Linseed oil (liquid)	1 48 (higher when solid)
Copal (varnish resin)	1 528
Rosin	1 548
Gelatin	1.530
Casein	1 675
Pigments of low opacity	
Silica	1.55
Whiting	1 60
Barium sulfate	1 64
Pigments of high opacity	
Basic lead carbonate	1 94 to 2.09
Zinc oxide	2.02
Very opaque pigments	
Zinc sulfide	2.37
Titanium dioxide	2.76

In a nonporous coating of linseed oil paint, whiting contributes practically nothing to the opacity because its index of refraction is about the

same as that of dried linseed oil. The opaque pigments, however, though less opaque than the dry powders in air, are opaque when embedded in a coating of dry linseed oil. Whiting in a nonporous coating of casein paint or glue-bound calcimine would likewise prove transparent because casein and glue have about the same index of refraction as whiting. In porous coatings, on the other hand, the presence of air-filled voids in the coating creates many whiting-air and vehicle-air interfaces with large differences in refractive index as a result of which the coating becomes opaque. When the coating can be made sufficiently porous, therefore,

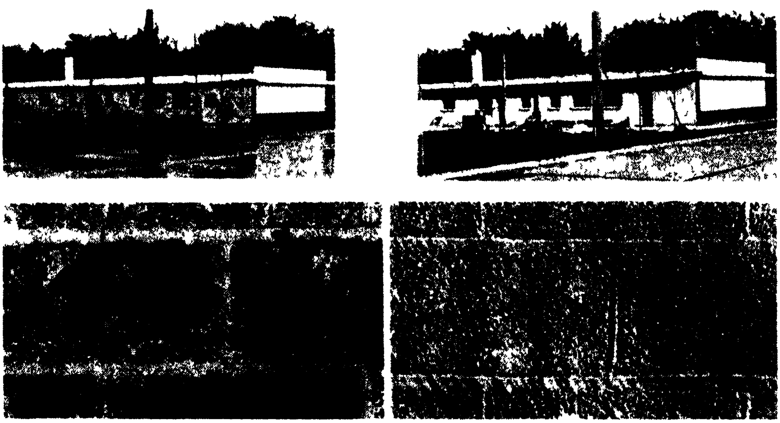


Figure 47.—Porous paints lose most of their opacity when they are thoroughly wet. The rear wall of the building shown is constructed of cinder block painted with a water paint that leaves a porous film. The views at the left were taken during a rain-storm, those at the right about an hour after the rain had ceased and the sun had come out.

pigments of low opacity such as whiting provide opacity as effectively as do the more expensive opaque pigments in nonporous coatings.[28a, 31]

The opacity of porous coatings made with pigments of low opacity does not develop until the volatile thinner has evaporated and the coating is dry, because prior to that time, the voids between particles of pigment are completely filled with liquid rather than with air. If after drying the coatings again become saturated with water or other liquid, as happens with exterior coatings during rainstorms, the opacity becomes seriously impaired until the imbibed liquid evaporates once more. A practical example of loss of opacity of an exterior coating of porous paint during a rainstorm is shown in Figure 47.

The development of the calcimines and casein paints as inexpensive decorative coatings may be attributed chiefly to the fact that they can be made with a very low ratio of nonvolatile vehicle to pigment[27] and

therefore make very porous coatings in which such pigments as whiting are opaque. Of course if the nonvolatile vehicle is too greatly reduced the pigments are not adequately bound and "chalk" when the coating is rubbed. Flat wall paints with oil or varnish vehicles require a much higher ratio of nonvolatile vehicle to pigment to prevent chalking,[25] and therefore cannot be made as porous as casein paints and calcimines, and cannot so effectively develop opacity with pigments of low refractive index.[31] No one has yet explained why flat wall paints require more nonvolatile vehicle than casein paints; but differences in the rate of gelatinization of oleoresinous and aqueous jellies, in the strength of the jellies, and in their surface and interfacial tensions undoubtedly result in differences in the manner of distribution of the jellies within the voids between the packed particles of pigment in the dried coatings.

The calcimines and earlier casein paints commonly contained only pigments of low opacity and colored pigments, with none of the opaque white pigments. In the last decade or so, however, opaque white pigments have been used in casein paints of the better type, both powder and paste paints. The opacity of such paints is high enough to ensure adequate hiding of the underlying surface with one coat of paint; but in addition these pigments produce brilliant white paints of very high diffuse reflectance[22, 80, 118] and pastel tints of a clearness and softness of color rarely attainable in flat paints having oleoresinous vehicles.[22, 163] If made with suitable pigments such as magnesium oxide, casein paints provide high reflecting power for ultraviolet light,[131] which is of biological importance. These results presumably arise from the fact that most of the incident light is reflected before it has penetrated very deeply into the coating so that very little of it is absorbed in passing through pigments or vehicle except for the desired selective absorption by the tinting pigments.[22, 27, 182]

Competitive Position of Casein Paints

In both cost and utility casein paints stand between calcimines and oleoresinous flat wall paints.

Calcimine, sometimes spelled kalsomine, usually means a distemper in which animal glue or dextrine is used as nonvolatile vehicle and whiting as the principal pigment. It is sold in the form of dry powder to be mixed with water by the painter and is intended primarily for the decoration of plastered interior walls. Some calcimines must be mixed with hot water, others with cold water, and still others with either hot or cold water. Calcimine represents the least expensive decorative coating available above the crude level of whitewash. It makes flat coatings with clear, soft colors that do not fade and do not turn yellow in subdued light. It is quickly applied with wide brushes and preliminary sizing of the surface is usually unnecessary, making the cost of application low. It

dries quickly and without the lingering odors of volatile thinners and oxidation products of oil paints, so that rooms can be redecorated quickly. It can be recoated several times, though with some difficulty, so that the best practice is to wash off the old coating before repainting. Calcimine, however, is readily stained or damaged by water and is suitable only for places that are always dry. When it becomes soiled it cannot be washed but must be recoated or removed and replaced. Some users consider that the lack of washability is not entirely a disadvantage because calcimine is easily removed by washing, permitting the surface to be kept decorated without accumulating a coating of many layers, which in time leads to difficulties with paints of all kinds.

Washable calcimine can be made in a number of ways: with a combination of glue and paraform (paraformaldehyde), with sodium silicate and starch, with albumin and an aldehyde; but the simplest and most economical way is by substituting casein and lime for the animal glue. Most of the washable calcimines on the market are casein paints of this kind in which the proportions of casein and lime are relatively low. Heaton[91] gives the following typical formula: Mix 2 parts of paris white (whiting), 1 part of barytes, and necessary colored pigments with a small proportion of linseed oil in an edge runner; then grind 70 pounds of this mixture to a fine powder with 8½ pounds of casein, 5 pounds of lime, and ½ pound of borax. One of the paints on the market in the United States contains 75 pounds of whiting, 10 pounds of kaolin, 6 pounds of lime, 4 pounds of casein, 4 pounds of dextrine, 8 ounces of trisodium phosphate, 1 pound of ultramarine, and a trace of beta naphthol.

On the more expensive side of the modern casein paints stand the oleoresinous wall paints, enamels, and cellulose ester lacquers for interior use, and exterior oleoresinous or oil paints for use on concrete, stucco, and brickwork. These exterior paints may be comprised for the present purpose in the collective term oil paints. The oil paints were well established commercially and had attained a fairly stable sales equilibrium with the calcimines before the modern casein paints appeared. The newer casein paints are still gaining ground rapidly at the expense of both calcimines and oil paints[61, 72] and there is consequently much interest in and difference of opinion about the relative advantages and disadvantages of the casein paints.[16, 25, 27, 76, 80] When equilibrium is again reached there probably will be found a substantial demand for all three types.

In comparison with oil paints the main advantages of modern casein paints are: (1) lower cost, particularly since one coat without sizing* or

* Some authorities consider that it is always advisable to size plaster walls before applying casein paint. If the plaster is unevenly absorptive because of non-uniform texture, patched areas, or cracks, sizing is advisable. Much casein paint, however, has been applied satisfactorily on plaster walls without first sizing them.



Computing room, where tinted paint on the plaster walls and white paint on the concrete and Pyrobar ceiling reduces the glare from sunlight on bright days and improves illumination on dark days.



Timber Mechanics laboratory, where white paint on concrete and Pyrobar ceiling and on hollow tile and brick walls gives good illumination even in parts of the room distant from windows



Paper Testing Room, which is an interior room with no windows and kept at 65 per cent relative humidity; casein paint is used on the hollow tile walls and concrete ceiling, oil paint on the woodwork.

Figure 48.—Appropriate uses of casein paints for interior decoration and control of illumination at the Forest Products Laboratory.

other preparation of the surface is practicable with casein paint but not with oil paint; (2) less time required to complete the job because of fewer operations, use of wider brushes, rapid drying, and freedom from lingering odors; (3) greater assurance of obtaining a dead flat surface with high diffuse reflection, freedom from "shiners" (glossy streaks caused by inept application), clear, soft colors in pastel tints, and freedom from yellowing in subdued light; (4) applicability to new plaster that is not yet thoroughly dry without danger of blistering, scaling, or "alkali burning" (saponification of the nonvolatile vehicle by the action of alkali and water); and (5) less rapid collection, less tenacious retention, and easier washing off of superficially deposited dirt of a solid nature, such as soot, dust, or pencil marks. On the other hand oil paints have the following advantages over casein paints: (1) availability in a range of surface sheen from flat to brilliant gloss; (2) protective power against rapid passage of moisture and consequent ability to protect metal against corrosion, wood against weathering, and concrete and stucco against absorption of free water; (3) less serious staining by and absorption of liquids or greases and greater resistance to the more vigorous methods of washing required to remove them; and (4) greater resistance to the growth of molds (mildew) and decomposition of the vehicle when exposed to warm, moist atmospheres.

Neither casein paint nor oil paint is as easy to remove as calcimine when the coating is worn out, but casein paints may be recoated with casein paint, and oil paint with oil paint. As many as 20 successful recoatings of casein paint are reported.[61] At times scaling occurs on repaint jobs with casein paint[76] but that is true with paints of all kinds, especially where there is an accumulation of many coats that vary more or less in composition. Where casein paint has been used for the first job on new plaster, for example, subsequent painting may be done with oil paint or lacquer,[34, 44, 126, 128, 132, 216] but a sizing or priming coat over the casein paint is then necessary.

In considering the competitive position of calcimine, casein, and oil paints it should be pointed out that neither glue nor casein makes cheaper paint than linseed oil except insofar as it makes possible the use of much less nonvolatile vehicle, more of the inexpensive pigments of low opacity, and more of the very inexpensive volatile vehicle, water.[25] Considering prices quoted for March 21, 1938 (*Oil, Paint and Drug Reporter*), linseed oil costs 70 cents, casein 95 cents, and calcimine grade of bone glue about $1.35 a gallon of "solid" bulk. The raw materials of the casein paint of Figure 45, however, cost 24.5 cents per gallon of paint ready for application, of which 6.3 cents is for casein; whereas the raw materials for the flat wall paint cost 64.5 cents a gallon of which 19.1 cents is for oil and resin. The costs of manufacturing, packaging, transportation, and distribution may be assumed to be approximately the

same for the two types of paint.[25] Modern casein paints in paste form sell at 20 to 30 per cent less than flat wall paints.[16] Nonporous casein paints with glossy surface for architectural uses so far have made no progress commercially because they would be more expensive than oil paints without having sufficient technical advantages over them. The greater hygroscopicity of casein necessarily puts it at a serious disadvantage in comparison with oil vehicles for making protective paints.

Powder, Paste, and Oil-Containing Casein Paints

Since the only liquid necessary in casein paints is water, the most economical way of distributing them is in the form of dry powders to be mixed with water by the painter.[80] Powder paints are also cheaper to manufacture than paste paints.[25] As long as casein paints remained merely washable calcimines they were always sold in powder form. When the modern casein paints using opaque white pigments appeared, however, their development split along two lines; one continued production and sale in the dry form and the other in the form of paste, in which there is enough water to hold the casein in solution but to which the painter adds more water, usually about half the volume of the paste. With some brands of paste paints the painter may add a mixture of lime and water. There is much difference of opinion about the relative merits of the powder and paste forms.[16, 25, 27, 61, 80] The question is fraught with difficulty, because, while there are fundamental technical differences between powder and paste paints, conflicting opinions are deeply colored by considerations of a purely commercial nature.

Proponents of the paste form argue that powder paints were so long associated in the public mind with calcimine, and were "considered as a means of selling ground whiting" by manufacturers,[25] that sale of a superior product at a necessarily higher price in the powder form was impracticable. Powder paints containing opaque white pigments in point of fact did not appear until paste paints had become established, and therefore powder paints have been called "cheap substitutes for the paste paints of high quality."[25] It is further argued that "the manufacture of a powder paint is essentially foreign to the plant activities of the average paint manufacturer" and "takes us back to the days when each painter ground his own paint."[25] On the other hand one manufacturer of powder paint summarized part of the opposing view in an advertisement, "water $24.00 per barrel—reasonable on a desert—outrageous in a paint."[165]

The business considerations are also largely influenced by the fact that the paste paints have been controlled since their origin by a patent license structure. Since the early days of the manufacture of mixed-pigment paints when "patent paints" acquired a bad reputation[92, pp. 11-33] the paint industry has been traditionally opposed to patent control of paint prod-

ucts, as distinguished from raw materials. The case for patent control has been stated as follows:[21] "From the beginning we have felt this a unique field where patent protection and the use of patents could benefit the industry as a whole. The manufacture and sale of flat wall paints has degenerated into a cut price proposition with relatively little opportunity for profit to the manufacturer. Casein paints will follow the same path except for the use of a patent license structure which is already in existence and has been accepted by a substantial number of large and important manufacturers. We have no thought of a monopolistic structure but have tried to build one solely for the benefit of the paint industry although, of course, a small royalty has to be charged in order to pay for the establishing and policing of such a structure." That there is much to be said on both sides of the question is indicated by the fact that most of the large manufacturers of casein paints make both powder and paste paints.[80]

For reasons arising from the physical chemistry of casein as described in Chapter 3 the technology of powder paints differs fundamentally from that of paste paints. In powder paints the casein vehicle during distribution and storage is in the dry form, though mixed with pigments, lime, and salts. On addition of water immediately before use it must dissolve promptly in cold water and with the simplest mixing equipment.* To accomplish the purpose the solution must be strongly alkaline, well to the alkaline side of the viscosity maximum of Figure 5. Furthermore, since the subsequent insolubility is brought about by the action of calcium hydroxide,[185] the pH must be in the region of maximum combination with bases indicated in the titration curves of Figure 4. In that region of pH, however, casein undergoes hydrolysis rapidly and calcium caseinate solutions of the necessary concentration coagulate to jellies within a few hours, unless hydrolysis proceeds too far in the meantime. The working life of casein powder paints is longer than that of casein glues (Chapter 8) because the concentration of casein is less and the higher content of dispersed solids, pigments and insoluble salts of calcium retards gelation. The powder paints should be mixed with water and applied the same day. Where practicable they are mixed to best advantage in power-driven mixers such as bakers' dough mixers. During storage of casein powder paints it is essential that they be kept dry and well protected from dampness of any kind.

In paste paints the casein must remain in stable solution without change in viscosity, hydrolysis, or attack of microörganisms during distribution and storage. It is therefore necessary to keep the solution of casein well on the acid side of the viscosity maximum of Figure 5, usually in the flat region of the viscosity-pH curve, and in a region of pH where the titration curves of Figure 4 show that there is rela-

tively little combination of casein with calcium hydroxide or other bases. The paste paints can contain only limited proportions of calcium hydroxide, if any, because calcium hydroxide at the pH in question is changed to more highly soluble salts, and the casein is coagulated by the divalent calcium cations so formed. Hydrolysis of the casein is minimized by keeping the pH within the range of maximum stability and the concentration of casein high, and the attack of microörganisms is prevented by addition of suitable toxics. A reasonable degree of water resistance results from the slow rate of solution of casein in nearly neutral solutions. Properly manufactured paste paints are not damaged by freezing when shipped in cold weather, repeated freezing in "Dry Ice" leaving them uninjured.[80] While properly formulated paste paints remain stable in the package for a reasonable period of time, they are not stable indefinitely and should be used within a few months after manufacture, extravagant claims to the contrary notwithstanding.[61]

Drying oils are readily emulsified in casein paints. They are incorporated in some paste paints by the manufacturer. Some manufacturers of powder paints advise the painter to add linseed oil after the powder has been dissolved in water. The paint then contains an emulsion of the oil-in-water type and represents an intermediate stage between the casein paint and the flat wall paint of Figures 45 and 46. The coating produced is less porous than that of oil-free casein paint and there is a loss of opacity and clarity of color proportional to the reduction in porosity of the coating.[31, 164] If the paint contains much pigment of low opacity the loss of hiding power is serious and may require application of another coat of paint. In addition the paint is likely to acquire the property of yellowing in subdued light.[22, 184] The advantage gained from addition of oil presumably is more satisfactory resistance to washing, durability on exterior exposure, and, in the case of paste paints, perhaps an improvement in stability in the package.

"Washability" of Paints

The importance of resistance to washing is often overrated. Even where washable paints have been used, washing of painted surfaces is the exception rather than the rule. One manufacturer of casein paint estimates that 90 per cent of the surfaces for which casein paint is advantageous will never be washed.[25] Washing seems easy and inexpensive on first thought but in practice it often proves more difficult and costly than was anticipated, regardless of the kind of paint. At best a washed surface is rarely as satisfactory in appearance as a freshly painted one and where labor must be hired to do the washing it often proves just about as cheap to repaint.[80] Probably the chief advantage of washing once or twice between paint jobs lies in the fact that a longer time is required to build up an unduly thick coating of uncertain behavior.

When repeated and vigorous washing of a painted surface is required or where liquids or greases are very likely to be spattered upon the surface the nonporous oil paints have advantages that so far cannot be equalled by casein paints. To attain the highest resistance to washing and to staining by liquids the advantages of porous coatings in the way of appearance must be sacrificed. Flat wall paints of the oil type are marred to some extent by too vigorous or repeated washing and tend to become more glossy, but they are usually more resistant to washing than casein paints.[78] Inherently the drying oils and oleoresinous varnishes are less softened by water and less reactive with mild alkalies than casein, even when the casein is thoroughly hardened with formaldehyde. It should also be noted that both casein paints and oil paints require time for thorough hardening before they acquire their maximum resistance to washing. At least 30 days should elapse after application before paint of any kind is washed.[78]

Ordinary dirts of a solid nature commonly cling more tenaciously to oil paints than to casein paints. Methods of washing involving more or less scrubbing, which have long been found suitable for oil paints, cannot be used with casein paints; but on the other hand casein paints can often be cleaned by a mild rinsing, involving no scrubbing, that would be ineffective with oil paint. A distinction has therefore been drawn between "scrubability" and "washability"[25, 61, 80] according to which casein paints are not scrubable but are washable. Powder paints can be made to withstand more vigorous washing than paste paints but the latter tend to hold dirt less tenaciously and therefore need less vigorous washing. The correct technic of washing casein paint is a light sponging of the surface with plenty of water and a small amount of mild soap, preferably with 2 or 3 per cent of 40 per cent formaldehyde solution in the wash water.[25, 182]

Raw Materials for Casein Paints

Opaque White Pigments

Technologically any of the pigments commonly used in oil paints may be used in casein paints, but in practice choice is made from a narrower group. The chemically active, basic pigments white lead and zinc oxide, which are very important in making oil paints, usually have no particular advantages in casein paints. White lead, because of its high specific gravity and consequent tendency to settle, as well as difficulty in dispersing it in water, is probably unsuitable and also unduly expensive, although its use is sometimes reported in patents.[114] Zinc oxide is likewise unnecessarily heavy and expensive, but it is sometimes used in the belief that it makes the paint harder.[3, 114, 150] The zinc sulfide pigments and the titanium pigments are lighter in weight and cheaper in terms of opacity per pound or per gallon in paint and are therefore

the principal opaque white pigments used for casein paints. Special grades of zinc sulfide pigments and of titanium pigments are made for use in casein paints, differing from the grades made for oil paints primarily in ease of dispersion in water.

The principal zinc sulfide pigments are lithopone, in "regular" and "double strength," zinc sulfide-magnesium pigment, and zinc sulfide. Regular lithopone consists of 28 per cent zinc sulfide and 72 per cent barium sulfate, coprecipitated from chemically equivalent solutions of zinc sulfate and barium sulfide, and the product calcined. Double-strength lithopone contains enough added zinc sulfide to make the zinc sulfide 50 per cent of the pigment. Zinc sulfide-magnesium pigment is a mechanical mixture of zinc sulfide and magnesium silicate (fibrous talc) in which the zinc sulfide amounts to 50 per cent. In all these pigments zinc sulfide is the opague ingredient; but it is so much more opaque than white lead or zinc oxide that regular lithopone and zinc sulfide-magnesium pigment may be considered fully equal to white lead or zinc oxide in opacity, and double-strength lithopone definitely superior.

The principal titanium pigments are titanium-barium pigment, titanium-calcium pigment, titanium-magnesium pigment, titanium dioxide, and lead titanate, the last a heavy pigment not used in casein paint. Titanium-barium pigment is of two types, one containing 25 per cent and the other 30 per cent titanium dioxide precipitated in the presence of freshly precipitated barium sulfate, and the mixture calcined. Titanium-calcium pigment is similarly made with 30 per cent titanium dioxide and 70 per cent calcium sulfate. Titanium-magnesium pigment is a mechanical mixture of 30 per cent titanium dioxide and 70 per cent magnesium silicate. Titanium-barium (25% TiO_2) pigment is fully as opaque as regular lithopone, and titanium dioxide is even more opaque than zinc sulfide. There are also titanated lithopones which are mechanical mixtures of approximately 15 per cent titanium dioxide with regular lithopone.

Pigments of Low Opacity

When the volume of pigment desired in paint exceeds the volume of opaque pigment considered necessary for opacity, the difference is made up with inexpensive pigments, which are all of low opacity. This simple statement of fact has long been considered too technical for the public to understand. Consequently many magic properties have been attributed to the inexpensive pigments for the purpose of explaining their presence in paint. It is true, of course, that both settling of paints in the package and working of paints under the brush depend upon their consistency, which in turn depends both on the total volume of pigments and certain characteristics of the individual

pigments; but consistency can be controlled when necessary without recourse to pigments of low opacity.

The principal pigments of low opacity used in casein paint are probably whiting (calcium carbonate) and kaolin (white china clay). There are a number of types and grades of whiting. English chalk, the finest grade of which is known as paris white, is preferred for calcimines and casein paints. Paris white should be so finely ground that 99 per cent will pass through a 300-mesh sieve. A medium grade of chalk is known as gilders' whiting and still coarser grades are used in putty. Dry, ground limestone and precipitated whiting are also used to some extent.

Other pigments of low opacity suitable for casein paints are magnesium silicate (fibrous talc), barium sulfate (blanc fixe and barytes), silica, diatomaceous earth, and calcium sulfate.[169, 181] Flake pigments such as mica[3, 26, 46, 175, 177] are said to minimize danger of checking or cracking of the coating. According to patent claims unusual pigments such as ground mollusk shells,[55] volcanic ash,[33] and a colloidal clay known as bentonite[102, 134] are useful for one purpose or another. Paints for special purposes sometimes require special pigments to produce desired physical modifications of the coating; among such pigments are asbestos,[177] granular pumice,[171] sawdust and cork,[99] magnesium oxide, magnesium carbonate, and alumina.[131]

Mica and calcium carbonate are sometimes coated with a very thin, possibly monomolecular layer of oleic acid, stearic acid, or resin to promote ready dispersion and high pigment concentration without unduly high consistency[58, 153] or they may be specially prepared in other ways.[122, 205]

The pigments of low opacity for use in casein paints should be free from heavy metals or water-soluble electrolytes.[124, 183]

Colored Pigments

The colored pigments for casein paints should be lime-proof, that is, nonreactive with alkalies.[127, 132, 181, 208, 223] In powder paints the vehicle is alkaline enough to change the color of pigments sensitive to alkalies; and paste paints, though more nearly neutral in reaction, should be capable of application to "green" plaster, concrete, or stucco on which they may be subject to the action of alkalies. Most of the colored pigments used are inorganic substances, either minerals or manufactured products. A few of the lime-proof lake pigments made with organic dyes may be used for brighter colors in casein paints.

The principal red pigments are the natural and artificial iron oxide pigments. Iron oxide reds range from nearly pure hematite (Indian red) and manufactured ferric oxide through the Spanish and Persian oxides, containing 75 to 85 per cent ferric oxide, to the Venetian reds,

made by calcining ferrous sulfate with whiting or by grinding calcined ferrous sulfate (Turkey red) with calcium sulfate (terra alba) and containing 8 to 40 per cent iron oxide. The iron oxide pigments yield a wide range of reds but all are rather dull. Tuscan red is iron oxide brightened by an organic lake such as alizarine lake, which is fast to lime. For bright reds lithol red, paratoluidine red, which are fast to lime, and paranitraniline red, which is less expensive but not quite so fast to lime, are used in the form of lake pigments. The cadmium reds (cadmium lithopones) are also suitable.

The iron oxide yellows are fast to lime and suitable for casein paints. The coloring matter is hydrated ferric oxide. The nearly pure oxide is available in manufactured colors. The earth colors, ochre and raw sienna, are also used widely. For brighter yellows zinc chromate, lead antimonate (Naples yellow), cadmium sulfide, cadmium lithopone, and Hansa yellow are suitable. Although the chrome yellows are sometimes used[39, 86] they are not fast to lime.[100] On the walls of the room in which this is being written a casein paint tinted cream with light chrome yellow has turned pink, but the same paint tinted with iron oxide yellow retains the original color. Orange chrome (basic lead chromate) may be used safely in casein paints.

For green, chromium oxide is lime-fast but rather dull in color. Green earth, a mineral of European origin, is used, particularly as a base for green organic lake pigments. The chrome greens and zinc greens are unsuitable because they are not fast to lime. Malachite green lake pigment is suitable for bright green.

For blue, ultramarine and the lime blues are widely used. Lime blue may mean ultramarine mixed with calcium sulfate or an organic lake color such as methylene blue lake. Indigo blue and cobalt blue (smalts) are also suitable. The iron blues are not fast to lime. The new copper phthalocyanin (Monastral fast blue) lake pigments[14, 93, 119] have come into use recently and are lime-fast.

For brown the iron oxide pigments, both the earth colors such as burnt sienna, raw and burnt umber and manufactured iron oxides and manganese brown are suitable.

For black the various carbon blacks, black oxide of iron, and manganese dioxide are used.

Aluminum bronze powder is mentioned in patents as a pigment in casein paints[12, 96] but the vehicle must not be too strongly alkaline.

Kind of Casein

In the manufacture of casein paints in the United States preference has been given to the caseins of domestic manufacture although some Argentine and Australian casein is used. Sulfuric-acid or hydrochloric-acid casein is often found more satisfactory than lactic-acid or grain-

curd casein because a casein of fairly high viscosity proves desirable. The concentration of casein in solution in casein paint ready for application is relatively low, about one-fourth that in casein glue, for example, yet a vehicle of reasonably high viscosity is necessary to make a paint with satisfactory brushing qualities. Reasonable uniformity in viscosity from shipment to shipment is even more important. It is convenient to specify that the viscosity of a 10 per cent solution of the casein in 15 per cent aqueous borax should agree with that of a similar solution of a control sample of casein.[40, 89]

Casein for use in powder paints must be finely ground and bolted. Most of it should pass through a 100-mesh sieve. Casein for use in paste paints is more conveniently purchased in granular form. The casein should be clean, free from rancid or sour odor, with a moisture content not exceeding 9.0 per cent, ash not exceeding 4.0 per cent, and fat less than 1.0 per cent.[146, 203, 223]

The solubility of the casein is very important, particularly so for casein paste paints. The casein should be completely soluble in 15 per cent aqueous borax or in some other solubility test agreed upon between buyer and seller.

Stability of the casein and freedom from decomposition, infestation by insects or microörganisms is important both for powder and for paste paints. The Warth incubation test described in Chapter 6 should be of value in this connection.

Chemical Reagents

The hydrated lime for use particularly in casein powder paints should be purchased under specifications to ensure uniformity in character.[223] It should be made by slaking fresh, well burned quicklime with about a third of its weight of water. It should be a white, amorphous powder, sufficiently fine to pass a 100-mesh sieve, and should be packed in moisture-proof, lined barrels or air-tight containers to prevent deterioration in transportation or storage. The sum of calcium carbonate and magnesia should be less than 4 per cent and iron oxide should not exceed 0.30 per cent. A hydrated lime of the following typical analysis is acceptable:

Total lime,	CaO	70.44 per cent
Magnesia,	MgO	1.50 " "
Silica, iron oxide, and alumina,	SiO_2, Fe_2O_3, Al_2O_3	2.40 " "
Carbon dioxide,	CO_2	1.40 " "
Combined water,	H_2O	24.09 " "

In casein powder paints additional reagents besides lime are required to increase the alkalinity of the solution when the powder is added to cold water in order to hasten solution of the casein and retard the coagulating action of the excess lime. The chemistry involved is similar

to that for casein glues, described in Chapter 8. In general any alkali metal or ammonium salt of an acid whose calcium salt is less soluble than calcium hydroxide will exert the desired action by releasing the corresponding hydroxide when the mixture is added to water. When the painter makes his own paint on the job, which is rarely done in the United States, alkali metal or ammonium hydroxides or sodium silicate may also be used. In paste paints, in which the casein is dissolved by the paint manufacturer—a process which may require heating—a still longer list of reagents becomes practicable, and alkali metal or ammonium salts of weak acids, which hydrolyze in aqueous solution, as well as organic bases may be used.

Common reagents are borax,[3, 24, 176] sodium fluoride,[24, 103, 104, 166] ammonium fluoride,[103, 104] trisodium phosphate,[48, 86, 103, 104] ammonium hydroxide,[83, 105, 154, 190] sodium hydroxide,[154] sodium carbonate,[2, 157, 191] sodium abietate or oleate,[124] and sodium silicate.[9, 144, 151, 192, 220] Other reagents mentioned in patents are ammonium and alkali metal metaphosphates[2, 112, 180] and pyrophosphates,[112] sodium fluoborate,[59] sodium tungstate,[151] calcium aluminate,[129] ammonium or sodium thiocyanate,[87] and organic bases such as urea,[51, 65, 87, 123, 206] triethanolamine,[51, 87] ethyl amine, piperidine, and piperazine.[103, 104]

The lime in casein paints is sometimes called an insolubilizer because it is responsible for coagulation of the casein in an insoluble form. Formaldehyde likewise makes casein insoluble but, unless the proportion used is too small to be very effective, it reacts too rapidly to be used in paints, though its use is reported.[40, 51, 90, 198] The polymer of formaldehyde, paraformaldehyde (paraform) reacts less rapidly in neutral and acid solutions and has been used to make animal glue water-resistant,[37] but in alkaline solution it reacts too rapidly. Another polymer, trioxymethylene, with an organic anhydride to suppress premature coagulation, is patented.[201] The compound of formaldehyde with ammonia, hexamethylenetetramine (hexamine), reacts slowly in alkaline solutions and can be used in powder paints to the extent of 5 to 8 per cent of the casein,[5, 65, 86, 167] but is usually considered too expensive. Its reaction is further retarded by ammonium hydroxide or ammonium salts but is accelerated by acids or by heat. Formamide,[137] aldehyde ammonia, and furfural have a similar action.

Chromates are said to make casein insoluble when acted upon by light.[40, 145] Similar action is claimed for ammoniacal solutions of heavy metal hydroxides such as copper hydroxide,[178, 179] many of which are strongly colored; but those of tin and zinc are free from this objection.

The insolubilizer is sometimes added by the painter when the paint is being mixed with water for application in order to avoid difficulty with premature reaction while the paint is in the package.[3, 69, 86]

Other Ingredients of the Nonvolatile Vehicle

In many of the powder paints dextrine is substituted for part of the casein for the purpose of increasing the viscosity of the vehicle and improving the consistency of the paint. Soluble starch to the extent of 2 per cent of the powder may be used for the same purpose.[83, 113, 137, 171] Dextrine and starch, however, impair water-resistance and resistance to washing.[86] Another purpose of dextrine is to plasticize the casein, making the paint less likely to crack and peel.[90] Other plasticizers used for the purpose are glycerol,[40, 85, 113] glucose,[40] sorbitol,[51] castor oil,[102, 137] and sulfonated castor oil.[86, 97, 141, 168] Rubber latex[62, 194, 198] and rubber derivatives[75, 196, 211] are used to the same end and, in larger proportions, to make exceedingly flexible paints.[160]

According to patent claims many other substances properly classifiable as part of the nonvolatile vehicle may be added to casein paints for one alleged purpose or another. Among these are paraffin wax,[83, 158] vegetable wax or Japan wax,[3] stearine,[83] ceresin,[73] beeswax,[73, 221] natural gums such as gum arabic and gum tragacanth,[38, 41, 204] rosin[143, 160] natural resins,[136, 179] oleoresins,[113] shellac,[222] phenolic resins,[86] alkyd resins,[105] soaps,[134, 140, 154, 179, 190] algin,[121] lecithin,[142] wool fat,[108] and water-soluble cellulose compounds.[179] Many of the patents, of course, seem to be based on the principle that anything that can possibly be incorporated might conceivably prove useful some day, whether it has any present value or not. It is even proposed to recover casein for paints from wastes in the casein plastics industry.[58]

Anti-foaming Agents, Preservatives, and Volatile Thinners

In dissolving powder paints and in thinning paste paints thorough stirring is necessary, during which there may be a tendency to develop foam which may then be stabilized by the pigments, particularly lithopone, and produce pin holes in the coating. Pine oil to the extent of 1 to 2 per cent is often used to prevent foaming but has the objectionable properties of imparting odor to the paint and irritating the eyes. Octyl alcohol is somewhat less effective than pine oil but is less odorous and irritating. Diethyl phthalate in the proportion of ½ to 2 per cent of the powder or paste is as effective as pine oil and is free from odor, though its homologues dimethyl and dibutyl phthalate are ineffective.[107] Liquid tertiary amines are also said to be effective.[74]

Preservatives and insecticides are sometimes used in powder paints, and preservatives are essential in paste paints to prevent attack by microörganisms.[127, 208] They may also be added for protection of the coating against mildew while in service.[81] It is apparently not always realized that a good preservative against bacteria, for example, may be of little use against fungi, or vice versa, and that a preservative may be effective against some fungi and ineffective against others. Con-

flicting opinions about the different preservatives may arise from failure to recognize their selective action.

Zinc sulfate was one of the earlier preservatives used in casein paints. If used to an extent greater than 0.25 per cent of the casein it tends to act adversely in the package, making powder paints cake badly. Mercuric chloride is too poisonous for paints containing it to be used generally. Sodium fluoride is fairly effective in proportions of 1 per cent of the casein or more.[7] Sodium silicofluoride is also said to be effective.[81] Alumino-fluosilicic acid in 25 per cent aqueous solution has been proposed as a sterilizing wash to be applied to the surface before painting, or to be added at the time of mixing to the extent of 2 per cent of the water used.

Organic preservatives are commonly preferred at the present time. Among them are beta naphthol,[6] sodium salicylate (1 per cent of the casein),[51, 86] thymol,[75] sodium orthophenyl phenate (0.25 per cent of the casein),[6] phenol,[7, 10, 15, 140] cresol,[43, 86] parachlor metacresol (0.1 to 0.15 per cent),[7, 10] benzyl phenols and cresols,[109] chlorinated phenols,[90] tribrom phenol,[176] pine oil (1 to 2 per cent),[86, 107] phenolic resins,[86] chlorinated naphthalene,[111] parachlor phenol, lead phthalate,[81] borosalicylic acid,[10] mineral oils containing sulfur,[189] and numerous proprietary products.[81] There is apparently marked difference of opinion about the relative effectiveness of the different preservatives; Harrison[89] lists in order of decreasing effectiveness, chlorinated phenols, thymol, beta naphthol, nitrobenzene, salicylic acid, cresylic acid, phenol. Camphor is mentioned as an insect repellent.[151]

The volatile vehicle in casein paints is nearly always water. Because water is cheap there is, of course, little incentive to use anything else, but other volatile liquids such as ethyl alcohol can be used in making casein paints for special purposes.[66, 191] Mixtures of organic liquids and water may be used as a means of reducing viscosity, acetone and ethylene glycol butyl ether being mentioned as particularly suitable for the purpose.[106] Others mentioned include butanol, diethylene glycol monoethyl ether, dioxane, ethyl acetate, ethyl lactate, and cyclohexanol.[105]

Manufacture of Casein Paints

Powder Paints

In powder paints it is essential that the casein be very finely ground so that it will dissolve reasonably quickly when the powder is added to water. The paint manufacturer usually grinds or regrinds the casein and sifts it, returning the tails to the pulverizer. The reagents, pigments, plasticizing materials, and preservative are then added in correct proportions and incorporated thoroughly in a dry mixer. The mixer has a set of revolving curved blades and is similar to the dry mixers sometimes used for blending pigments in the manufacture of oil paints.

After mixing the batch is passed through a stone mill or a high-speed pulverizer. It is then sifted again and the overtails sent back to the pulverizer until the whole batch has been reduced to a satisfactory degree of fineness; after this it is again mixed and packaged, usually in 5-pound cartons lined with moisture-proof paper of which either 10 or 20 are put up in a case. Larger quantities are packed in boxes, kegs, or drums containing 25, 50, or 100 pounds.

More elaborate methods of manufacturing powder paints have been described. The casein may be brought to a moisture content of 25 to 32 per cent, mixed with sufficient readily hydrolyzable salt of an alkali metal to form a "soluble casein," the mixture warmed and extruded in an apparatus similar to that used in making casein plastics, and then dried and ground before making powder paints in the customary way.[48] Another procedure is to dissolve the casein in a nearly neutral solution and then spray it into the violently agitated pigment after which the product is dried, ground, and bolted.[178]

The formula for casein paint already given, based on Federal Specification TT-P-23, is representative of the proportions of the major constituents of powder paints. The content of casein is usually 10 to 15 per cent by weight in powder paints of good quality. Correct ratio of calcium hydroxide to casein is essential because with too little calcium hydroxide the casein is not rendered sufficiently insoluble to make the paint washable, whereas too much calcium hydroxide coagulates the casein too quickly and makes the coating too brittle. The correct ratio depends to some extent upon the proportion of other reagents that affect the pH of the solution when the paint is mixed with water, but as a rule there should be 0.875 pound of CaO per pound of casein. If the lime used contains 70 per cent CaO the amount needed is 1.25 pounds per pound of casein, while a lime containing only 50 per cent CaO would have to be used in a ratio of 1.75 pounds per pound of casein.[200] Additional reagents to increase the alkalinity of the solution, such as trisodium phosphate, amount to 5 or 10 per cent of the weight of the casein. The balance of the powder, which is the major portion, consists of pigments except for minor additions of preservatives and agents to prevent foaming.

If 10 pounds of a powder paint containing 10 per cent casein, 12.5 per cent lime (70 per cent CaO), and 0.9 per cent trisodium phosphate is mixed with 1 gallon of water the trisodium phosphate promptly reacts with part of the lime, forming sodium hydroxide and calcium phosphate, and there are left 0.030 gram equivalent of calcium hydroxide and 0.0016 gram equivalent of sodium hydroxide per gram of casein. If it is assumed that calcium caseinate is formed to the extent of the so-called maximum binding capacity of casein, 0.0018 gram equivalent of calcium hydroxide per gram of casein, there is available more than

17 times the necessary amount of calcium hydroxide and the excess, amounting to 0.124 gram per gram of water added, is 100 times the solubility of calcium hydroxide in water at 20°C. It is probable that more than 0.0018 gram equivalent is bound per gram of casein, but in any event the amount of lime used is sufficient to make sure of an excess of calcium hydroxide present as a solid phase, so that both the casein and the solution are thoroughly saturated. The minimum pH in casein paints of this type is therefore that of a saturated solution of calcium hydroxide; but ordinarily it is still more alkaline by reason of the sodium hydroxide formed by reaction with trisodium phosphate or other similar reagent.

Paste Paints

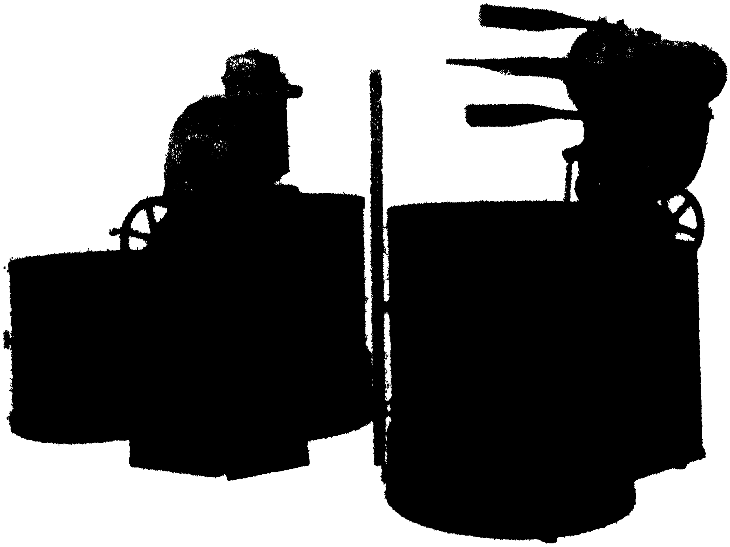
In the manufacture of paste paints casein is first dissolved in water, forming a solution whose viscosity must remain reasonably constant for several months. The pigments are then dispersed in the solution by grinding on a roller mill or other suitable mill just as pigments are ground in oil or varnish in making oil paints or enamels.

To obtain a solution of casein of sufficient concentration and in the restricted range of pH within which the viscosity is reasonably stable the casein must be dissolved carefully by an appropriate technic. The alkali-metal hydroxides are not convenient reagents for the purpose because the solutions formed are too strongly alkaline when sufficient casein has been dissolved to produce the required concentration. The difficulties should be clear from the discussion of the solubility of casein in aqueous alkalies in Chapter 3 The desired solutions lie in the region to the left of the solubility curve of Figure 3. Weaker alkalies such as ammonium hydroxide are more nearly practicable because the uncombined excess builds up the pH less rapidly. Solutions in the desired region can be obtained by dissolving the casein with alkali metal or ammonium hydroxide and then neutralizing the solution by adding a weak acid such as boric acid[24, 186] or by dissolving in warm solutions of alkaline salts such as borax or sodium fluoride,[87, 104, 157, 166, 190] in which casein swells considerably before going into solution. In the case of the alkaline salts, excess reagent fails to increase the pH unduly because of the buffer action of their hydrolytic equilibrium.

The method commonly followed in the United States is to heat the casein in warm aqueous solutions of suitable salts until it is greatly swollen and softened and then add sufficient weak alkali or alkaline salt to effect solution at a pH preferably between 6 and 7. According to an example given in Atwood's patents:[24] 55 pounds of casein is soaked in 30 gallons of water at 150 to 160°F., 5 pounds of sodium fluoride and 2 pounds of ammonium chloride are added, and the temperature maintained until the casein is greatly swollen, after which 6 pounds

of borax is added and the batch agitated thoroughly until solution is effected. The resulting solution is strongly pseudoplastic and at room temperature has a gel-like consistency.

Iddings[103, 104] claims that addition of amorphous silica, particularly diatomaceous earth, facilitates the formation of a "smooth" solution of stable viscosity. Just why it does so is not revealed but it is pointed



Courtesy of the Baker Perkins Company, Inc.

Figure 49.—A type of mixer used for mixing the pigments with the vehicle of casein paste paints. Both the paddles and the container, which is detachable, rotate to provide thorough agitation. Front and rear views, an extra container, and a 6-foot measuring stick to indicate dimensions are shown.

out in Chapter 8 that colloidal silica greatly increases the working life of casein glues made with sodium silicate and lime. Iddings gives the following example of his procedure: To 100 pounds of casein add 150 pounds of water, 14 pounds of sodium fluoride, 5 pounds of ammonium fluoride, and 7 pounds of silica; stir until the mixture is homogeneous and after the casein has imbibed water heat to 195°F., continuing the stirring; hold the temperature until a smooth paste results, which usually takes ½ to 1 hour (the longer the time the higher the viscosity of the resulting solution), and then dilute with 300 pounds of water at 120°F. The presence of the silica increases the pH slightly, for example, a batch without silica had a pH of 6.3 while an otherwise similar batch

containing silica had a pH of 6.9. By increasing the proportions of sodium fluoride and silica or by adding such alkaline salts as borax, sodium silicate, trisodium phosphate, or ammonia, or organic bases such as piperazine, the alkalinity of the solution may be increased.

The viscosity of the resulting solution of casein depends upon the nature and concentration of the casein, the time and temperature of heating, the pH, and the nature and concentration of the alkaline salts used. The viscosity may be further modified by addition of neutral



Courtesy of J. M. Lehmann Company, Inc. and the Wardway Paint Works

Figure 50.—A three-roller mill (in the foreground) of a type commonly used for grinding casein paste paints.

salts such as ammonium oxalate,[106] by addition of synthetic resins such as the ammonium soap of certain glycerol phthalate resins,[105] or by addition of organic solvents such as acetone, butanol ethyl acetate, dioxane, cyclohexanol, or diethylene glycol monoethyl ether.[106]

The equipment used for dissolving the casein may be much the same as that described in Chapter 9 on paper coatings. After the casein has been dissolved additional ingredients such as preservatives, anti-foaming agents, plasticizers, and modifying vehicles such as drying oils and resins may be added. The pigments are then mixed with the vehicle, usually in a change-can mixer of some type, such as that shown in Figure 49. Finally the batch is ground on a paint mill to incorporate the pigments with the vehicle thoroughly. For white and tinted paints a three-roller grinding mill like that shown in the foreground of Figure

50 is commonly used. Stone mills are sometimes used, particularly when the pigments are difficult to wet and disperse thoroughly in the vehicle, as is the case with some of the colored pigments.

After grinding casein paste paints the mills should be cleaned promptly and thoroughly and the iron rolls, scraper knife, and discharge chute greased to protect them from corrosion. The metal cans in which casein paste paints are packed must be made resistant to corrosion. It is believed that rust-inhibitive agents are commonly incorporated in casein paints to minimize difficulties with corrosion both of equipment and of containers but no information is available about the inhibitors used.

Exterior Casein Paints

Although the principal use of casein paints in the United States is for interior painting it is finding increasing application in exterior painting, chiefly on cement, stucco, cinder block, brick, or other surfaces on which a paint readily permeable to moisture is satisfactory. It furnishes no protection against weathering for wood and is therefore suitable only for rough or weatherbeaten woodwork. Casein paints made for interior use may not be durable out of doors.[82] In parts of Europe, particularly Germany, shortage of vegetable oils has led to increasing use of exterior casein paints.[8, 20, 162, 207, 209, 211, 219] In the United States interest in exterior casein paints was stimulated largely by their extensive use at the Century of Progress Exhibition at Chicago in 1933 and 1934, where flat paints were required for night illumination, and experimentation with new materials of lower cost was encouraged.[67]

Exterior casein paints must either contain a very substantial proportion of lime[11, 20, 86, 211, 219] or, as is more commonly the case, they must contain a more weather-resistant vehicle in addition to the casein. Most exterior casein paints sold in the United States are probably oleocasein paints, that is, paints containing substantial proportions of both casein and drying oil or varnish.[164, 197, 212] Tung oil is said to make more water-resistant paints than linseed oil.[164, 212] Oleocasein paints are also made from powder paints by adding linseed oil, in some such proportion as ½ pint to 5 pounds of powder, after mixing the powder with water. Another type of exterior paint containing casein makes use of a mixture of portland cement and casein as the binder.[114, 135, 147, 156, 187]

In oleocasein paints the oil is dispersed as droplets in the water vehicle and on drying the droplets of oil presumably coalesce, provided that there is enough oil present.[212] In a typical oleocasein paint there may be about as much drying oil as casein. Further increase in the ratio of drying oil to casein, beyond 2:1 for example,[209, 210] produces a type of paint known as an emulsion paint.[94, 124, 154, 173, 203, 208, 213] Solutions of synthetic resins may be used in emulsion paints in place of drying oils or varnishes.[1a] Emulsion paints are widely used in Europe both for in-

terior and exterior work but are unimportant in the United States. They contain a higher ratio of nonvolatile vehicle to pigment by volume and are correspondingly less porous than casein paints, and are sometimes made with enough nonvolatile vehicle to give a surface with "eggshell" gloss.[1a, 209] It is the writers' understanding that the European emulsion paints are considered ill-adapted in consistency and working qualities for application by painters trained in the United States and to meet the expectations of American property owners.

Casein Paints and Varnishes for Special Purposes

Because of the high ratio of pigment to nonvolatile vehicle attainable in casein paints they are suitable for plastic paints[23, 55] and because of their highly porous nature, for acoustical paints[23, 99, 171, 177] and for paints designed for high reflection of ultraviolet light.[131] Casein paints made with a high content of lime have been used for road-marking paints.[42, 86] Casein solutions emulsified in oleoresinous enamels or nitrocellulose lacquers are patented as a means of making mottled decorative paints.[45, 136] Casein is also proposed as an emulsifying agent in making thermo-setting linoleum composition.[188]

Casein is used in making artists' water colors,[23, 120, 221] for which it has the advantage of making more water-resistant paintings than water colors made with animal glue as vehicle. Casein colors need not be protected by a glazing coat or fixative and therefore retain their flat surfaces and clarity of color. For that reason the casein water colors are widely used for architects' sketches. Mildly alkaline solutions of casein and the finest grades of such pigments as zinc oxide, Naples yellow, ochre, cinnabar, madder lake, sienna, cobalt blue, Prussian blue, chrome green, and ivory black are used. Casein water colors are sold in soft paste form or in dry cakes made by evaporating water from the paste. In the latter case a hygroscopic agent such as diethylene glycol may be added to keep the cake from becoming too dry to dissolve promptly.

Glossy paints or enamels containing casein may be made by using a mixture of casein and the less expensive sodium or potassium silicate for the vehicle.[41, 115, 144, 192, 220] Semigloss paint can be made with casein and wax,[73] or with casein emulsion paints.[210] Silicate paints are fire retardants and additional fire-retardant chemicals such as ammonium salts and phosphates may be added to them, making paints suitable for such purposes as theatrical scenery.[41] Steel molders sometimes use a casein paint to spray on the surfaces of molds for the purpose of obtaining castings with clean surfaces.

Unpigmented casein coatings, or casein varnishes, are used for certain purposes. Such varnishes may be merely a mildly alkaline solution of casein[123, 152, 166, 170] or they may be mixtures of casein with natural resins,[178, 179, 180, 202] phenolic resins,[28, 141, 202] alkyd resins,[183] ethyl or

benzoyl cellulose,[161] cellulose acetate,[137] nitrocellulose,[60] or water-soluble compounds of cellulose such as Schweitzer's solution.[178, 179, 180] Both the casein and the resin or cellulose compound may be held in solution by a mutual solvent,[137, 141, 161, 178, 179, 180] for which purpose organic bases such as triethanolamine are particularly useful;[47] or the casein and the resin or cellulose compound may be dissolved separately and one solution emulsified in the other.[28, 133]

Casein varnishes are used as shellac substitutes,[141] for such purposes as coating textile bobbins,[86, 202] as coatings or dressings for paper, leather,[28, 161] or rubber,[84, 85, 152] to make cardboard containers impermeable to oils,[137] and to coat wooden containers for butter to prevent taint in the butter.[170, 214] They are said to be suitable as sealers on wood treated with creosote to prevent bleeding of the creosote into paint applied over it[133] and as primers for plaster walls over which architectural lacquers are to be applied[34, 216] or for wooden furniture to be coated with lacquer.[60] Probably the largest use of casein varnish has been as a final size for wall paper to make it washable,[166] as much as 10 tons of casein a month being used by a single manufacturer for that purpose.

Besides the emulsion paints already mentioned, in which casein is used in sufficient quantity to be considered an essential part of the nonvolatile vehicle, there are emulsion paints in which only enough casein to act as an emulsifying agent is used. Emulsions of bitumens,[49, 97, 130] alkyd resins,[110, 155] phenolic resins,[38, 50, 102] and nitrocellulose lacquer[95] are stabilized by mildly alkaline solutions of casein. Aqueous emulsions of these kinds have higher viscosity and less tendency to penetrate into porous materials like paper, leather, textiles, and wood than the ordinary solutions in organic solvents; the emulsions therefore often make more economical or more satisfactory coatings for porous materials. Casein is used also as an emulsifying agent in making aqueous emulsions of oils or metallic soaps of suitable viscosity and penetration for various industrial purposes; for example, mineral oil emulsions stabilized by casein may be used in textile dyeing[215] and emulsions of a gasoline solution of aluminum stearate are patented for water-proofing rock wool fibers.[98]

Aqueous emulsions of casein with rubber,[204] chlorinated rubber,[168, 195] or rubber latex[15, 52, 62, 75, 77, 116, 160, 187, 194, 198] together with dyes or pigments are used to make unusually flexible coatings for paper, textiles, and leather.

Testing, Statistics

Methods of analyzing and testing casein paints of the kinds commonly used for interior painting in the United States are described in detail in Federal Specification TT-P-23,[69] which is obtainable from the Superintendent of Documents, Washington, D. C., for 5 cents (stamps not accepted), and for that reason need not be discussed here. For paints

containing mixtures of casein with other nonvolatile vehicles there are as yet no generally accepted methods of analysis and testing. Testing methods in use in Europe have been published by several authors.[17, 30, 54, 146, 148]

Until recently there were no authoritative statistics on the quantity of casein paints produced in the United States. The Bureau of the Census reported production of "cold water paints," "calcimines," and "plastic paints" but did not distinguish between casein paints and paints with other vehicles. The total of cold-water paints, calcimines, and plastic paints, calculated as a fraction of the total production of paste and prepared paints, was approximately twice as large in 1935 as it was in 1925. During the past decade the annual production of cold-water paints, calcimines, and plastic paints has amounted to roughly 125 to 150 million pounds, worth at the factory six million dollars. The total value of all products of the paint and varnish industries is about 400 million dollars. Of the total of cold-water paint, calcimine, and plastic paint about 60 per cent is calcimine, 35 per cent cold-water paint, and 5 per cent plastic paint.

At the present time the Bureau of the Census issues monthly statistics in which the casein-bound cold-water paints and plastic paints are distinguished from those bound with other vehicles. The reports are based on data furnished by 53 identical manufacturers. The following data are abstracted from the report released May 9, 1938:

Item	Total sales in pounds for the first 3 months (January–March) of 1938	of 1937
Plastic paints		
Paste, casein-bound	142,968	102,480
" , all others	75,982	93,261
Dry powder, casein-bound	699,657	839,670
" " , glue-bound	509,942	710,262
Cold-water paints		
Exterior, casein-bound	1,372,612	833,641
" , lime and/or cement-bound	1,348,888	1,527,303
Interior, casein-bound		
paste form	5,281,188	4,432,032
dry powder form	1,467,745	1,577,874
" , glue-bound	1,170,329	1,606,113
Calcimines		
Hot-water	9,481,621	12,023,666
Cold-water	8,965,710	9,975,427

The annual production of casein paint in the United States is probably in excess of 35 million pounds. Most of the well known brands of casein paint are advertised as conforming to Federal Specification TT-P-23,[69] which requires a minimum of 10 per cent casein in powder paints and 6.5 per cent in paste paints. The annual consumption of casein for

manufacturing casein paints, therefore, probably amounts to at least 2.75 million pounds. In addition, casein is used in most of the cold-water calcimines and in other paint and varnish products not segregated conveniently in the Census returns. The total annual consumption of casein in paint and varnish products may be as much as 3.5 million pounds out of a total annual consumption of 60 to 70 million pounds of casein.

Before casein paints appeared in the paste form the manufacture of calcimine and cold-water paints was more or less of a separate industry, distinct from the manufacture of oil paints and varnishes. The principal merchandising impetus still comes from those who are makers of water paints primarily, but at the present time many manufacturers of oil paints are manufacturing casein paints also.

References

1. Abrahams, I. B., U. S. Pat. 377,283 (Jan. 31, 1888)

1a. Albertsen, K., *Seifenseider-Ztg.*, **65**, 121, 137 (1938); *Progress Interpreted,* No. 76 (1938)

2. Albright and Wilson Ltd., Brit. Pat. 462,114; Abridg. Spec. 1937 Group IV, 70

3. Allen, W. B., U. S. Pat. 1,477,096 (Dec. 11, 1923) Reissue 16,175 (Sept. 22, 1925); 1,718,936 (July 2, 1929)

4. American Society for Testing Materials, Book of A.S.T.M. Standards, **1936II**

5. Anonymous, *Oil Paint and Drug Reporter,* **92,** No. 25, 32A (1917)

6. Anonymous, *Industrial Finishing Mag. (London),* **1,** 184 (1930)

7. Anonymous, *Farben-Ztg.,* **36,** 1571 (1931)

8. Anonymous, *Zementverarbeitung,* No. 27, Berlin (1931) (Zementverlag G.m.b.H.)

9. Anonymous, *Mat. grasses,* **23,** 9280 (1931)

10. Anonymous, *Tech. Mitt. Malerei,* **49,** No. 20, 166 (1933)

11. Anonymous, *Paint and Varnish Production Mgr.,* **10,** No. 3, 32 (1934)

12. Anonymous, *Rev. gen. Mat. Plast.,* **10,** No. 12, 456 (1934)

13. Anonymous, *Chem. Age,* **32,** 369 (1935)

14. Anonymous, *Paint Manuf. (London),* **5,** 368 (1935)

15. Anonymous, *Farbe und Lack,* **1935,** 342

16. Anonymous, *Industrial Bulletin, Arthur D. Little, Inc.,* No. 124 (May 1937)

17. Anonymous, *Paint Tech. (London),* **2,** No. 18, 200 (1937)

18. Anonymous, *Paint Tech. (London),* **2,** No. 14, 63 (1937)

19. Anonymous, *Decorator (London),* **36,** 51 (1938)

20. Asmuss, E. W., *Tech. Mitt. Malerei,* **53,** No. 15, 151 (1937)

21. Atlantic Research Associates, Open letter on the manufacture and sale of casein paints (not dated)

22. Atwood, F. C., *Amer. Paint and Varnish Mfrs. Assoc., Sci. Sec. Circ.,* **370,** 465 (1930); *Amer. Paint J.,* **14,** No. 52D, 25 (1930); *Paint Oil and Chem. Rev.,* **90,** No. 17, 80 (1930); *Oil Paint and Drug Reptr.,* **118,** No. 19, 54 (1930)

23. Atwood, F. C., *Amer. Paint J.,* **16,** No. 44, 15 (1932)

24. Atwood, F. C., U. S. Pat. 1,893,608 (Jan. 10, 1938); Brit. Pat. 414,044; Abridg. Spec. 1934 Group III, 237

25. Atwood, F. C., *Amer. Paint J.,* **18,** No. 19, 48, No. 21, 17 (1934); *Plastic Products,* **10,** 107 (1934); *Drugs, Oils and Paints,* **49,** 60 (1934)

26. Atwood, F. C., *Official Digest, Federation of Paint and Varnish Production Clubs,* No. 148, 328 (1935)
27. Atwood, F. C., *Amer. Paint J.,* **20,** No. 33, 16 (1936) ; *Drugs, Oils and Paints,* **51,** No. 6, 242 (1936) ; *Official Digest, Federation of Paint and Varnish Production Clubs,* No. 156, 192 (1936)
28. Bakelite Corpn., Brit. Pat. 422,416 (July 6, 1933)
28a. Baltimore Club, *Natl. Paint Varnish and Lacquer Assoc., Sci. Sect. Circ.,* **568,** 261 (1938) ; *Amer. Paint J.,* **23,** No. 5, 14 (1938)
29. Bancroft, W. D., "Applied Colloid Chemistry," New York, McGraw-Hill Book Co., 1921
30. Beck, K., *Gelatine Leim Klebstoffe,* **2,** 173 (1934)
31. Becker, E. A., *Farben-Ztg.,* **42,** 1163 (1937)
32. Bingham, E. C., *Amer. Chem. J.,* **46,** 278 (1911) ; *J. Franklin Inst.,* **181,** 845 (1916)
33. Binnington, D. S., Can. Pat. 303,051 (Aug. 12, 1930)
34. Bogin, C., U. S. Pat. 1,937,484 (Nov. 28, 1933) ; Pringle, W. L., U. S. Pat. 2,104,240 (Jan. 4, 1938)
35. Browne, F. L., *Ind. Eng. Chem.,* **25,** 835 (1933) ; **28,** 798 (1936)
36. Browne, F. L., *Ind. Eng. Chem.,* **29,** 1018 (1937) ; *Amer. Paint J.,* **22,** No. 1 20, No. 2, 32 (1937)
37. Browne, F. L. and Hrubesky, *Ind. Eng. Chem.,* **19,** 215 (1927) ; Hrubesky and Browne, U. S. Pat. 1,712,077 (May 7, 1929)
38. Bruckmann, K., Brit. Pat. 403,095 (Dec. 11, 1933)
39. Brusilovskii, A. M. and Tsarev, B. A., *Prom. org. Khim.,* **3,** 218 (1937) ; *Chemical Abst.,* **31,** 7268
40. Bryson, H. C., *Paint and Varnish Production Mgr.,* **8,** No. 4, 8 (1932) ; *Ind. Chemist (London),* **8,** 146 (1932)
41. Bryson, H. C., *Paint Manuf. (London),* **2,** 109, 222 (1932)
42. Bryson, H. C., *Paint Manuf. (London),* **4,** 205 (1934)
43. Bryson, H. C., *J. of Decorative Art,* **55,** No. 649, 17 (1935)
44. Building Research Station (London) Note No. 350 (Jan. 1931)
45. Busch, P., U. S. Pat. 2,091,466 (Aug. 31, 1937)
46. Callaghan, A. B., U. S. Pat. 1,787,425 (Jan. 6, 1931)
47. Carbide and Carbon Chemicals Corpn., "The preparation of emulsions and other products with triethanolamine," 1934
48. Casein Manufacturing Co. of America, Brit. Pat. 453,305; Abridg. Spec. 1936 Group IV, 412
49. Chemische und Seifenfabrik R. Baumheier A.-G., Brit. Pat. 379,076; Abridg. Spec. 1932 Group III, 328
50. Chemische und Seifenfabrik R. Baumheier A.-G., Brit. Pat. 385,344; 386,018; Abridg. Spec. 1933 Group III, 73, 81
51. Chibnall, A. C., Bailey, K. and Astbury, W. T., Brit. Pat. 467,812; Abridg. Spec. 1937 Group III, 144
52. Christmas, W. W., U. S. Pat. 1,724,906 (Aug. 20, 1929)
53. Church, J. W., *Official Digest, Federation of Paint and Varnish Production Clubs,* No. 124, 102 (1933)
54. Coffignier, Ch., *Rev. chim. ind.,* **28,** 399 (1919)
55. Crandell, D. D., U. S. Pat. 1,984,116 (Dec. 11, 1934)
56. Doormann, G., *Farbe und Lack,* **1930,** 254, 266, 279, 293, 304
57. Dropman, R. E., *Paint Manuf. (London),* **7,** 12 (Jan. 1937)
58. Dubovskii, G. B. and Averina, M. F., U.S.S.R. Pat. 50,207 (Jan. 31, 1937)
58a. Dunham, H. V., U. S. Pat. 790,821 (May 23, 1905)
59. Dunham, H. V., U. S. Pat. 1,347,845 (July 27, 1920) ; Brit. Pat. 164,604 (May 17, 1920)

60. Dunham, H. V., U. S. Pat. 1,922,936 (Aug. 15, 1933)
61. Dunning, S. C., *Paint Oil and Chem. Rev.*, **99**, No. 23, 21 (1937)
62. Dunstone, A. M., Belg. Pat. 389,394; *Chem. Zentr.*, **106 ii**, 2291 (1935)
63. Edwards, J. D., *Ind. Eng. Chem.*, **25**, 846 (1933)
64. Eibner, A., *Tech. Mitt. Malerei*, **53**, No. 4, 39 (1937)
65. Eisenstein, J., U. S. Pat. 1,852,706 (Apr. 5, 1932)
66. Etablissements Morin et Janet, French Pat. 800,905; *Chem. Zentr.*, **107 ii**, 3364 (1936)
67. Fawkes, C. E., *Plastic Progress*, **9**, No. 6 (Aug. 1933)
68. Federal Specifications Executive Committee, Spec. TT-P-46 (1931); superseded by Spec. TT-E-506 (1935)
69. Federal Specifications Executive Committee, Spec. TT-P-23 (1935)
70. Federal Specifications Executive Committee, Spec. TT-P-101a (1936)
71. Federal Specifications Executive Committee, Spec. TT-P-51a (1937)
72. Federation of Paint and Varnish Production Clubs, *Official Digest*, No. 173, 107 (1938)
73. Fessel, M., Czech. Pat. 52,815; *Chem. Zentr.*, **108 i**, 1566 (1937)
74. Flett, L. H., U. S. Pat. 2,074,380 (Mar. 23, 1937)
75. Freydberg, R. M., U. S. Pat. 2,052,393 (Aug. 25, 1936)
76. Fuller, W. R., *Ind. Eng. Chem.* special supplement on "Planning for 1937," Nov. 1936, p. 30 in adv. section
77. Galimberti, G. and Peverelli, G., Brit. Pat. 392,797; Abridg. Spec. 1933, Group V, 147; Ger. Pat. 613,906 (1935)
78. Gardner, H. A., *Natl. Paint Varnish and Lacquer Assoc., Sci. Sect. Circ.*, 508 (1936)
79. Gardner, H. A., "Physical and Chemical Examination of Paints, Varnishes, Lacquers and Colors," 8th ed., Washington, 1937
80. Gardner, H. A., *Amer. Paint J.*, **21**, No. 22, 25 (1937)
81. Gardner, H. A., Cornthwaite, C. R., and Hart, L. P., *Natl. Paint Varnish and Lacquer Assoc., Sci. Sect. Circ.*, **526**, 46 (1937)
82. Gardner, H. A. and Sward, G. G., *Amer. Paint and Varnish Mfrs. Assoc., Sci. Sect. Circ.*, **421**, 281 (1932)
83. Goodell, A. P. and Tarr, G. W., U. S. Pat. 1,700,404 (Jan. 29, 1929)
84. Goodyear Tire and Rubber Co., Brit. Pat. 366,160; Abridg. Spec. 1932 Group III, 115
85. Goodyear Tire and Rubber Co., Brit. Pat. 397,467; Abridg. Spec. 1933 Group V, 201
86. Hadert, H., *Farben-Chemiker*, **8**, 408, 421 (1937); *Progress Interpreted*, No. 73 (Mar. 1938)
86a. Hall, W. A., U. S. Pat. 651,851 (June 19, 1900)
87. Handelsvennootschap onder de firma Verceenigde Chemische Fabrieken "Ceta-Bever," Holland Pat. 41,105 (July 15, 1937)
88. Hanstock, R. F., *J. Oil and Color Chem. Assn.*, **20**, 5 (Jan. 1937)
89. Harrison, A. W. C., *British Plastics*, **2**, 78 (1931)
90. Harrison, A. W. C., *British Plastics*, **3**, No. 30 (1931); *Synthetic App. Finishes* (in *Paint Tech., London*), **2**, No. 20, 78 (1931)
91. Heaton, N., "Outlines of Paint Technology," Philadelphia, J. B. Lippincott Co. (1928)
92. Heckel, G. B., "The Paint Industry, Reminiscences and Comments," Amer. Paint J. Co., St. Louis, 1931
93. Heertjes, *Verfkroniek*, **9**, 191, 258 (1936)
94. Hengst, G., "Kaseinfarben und Kaseinemulsionsfarben und ihre Verarbeitung," Gallwey, Munich

95. Hercules Powder Company, *Oil Paint and Drug Reptr.*, **131**, No. 26, 12; *Farbe und Lack*, **1937**,; 537; *Hercules Pdr. Co. Circulars* 3 and 4 (1937)
96. Herve, F. R., Herve, M. and Herve, A., Brit. Pat. 253,889 (June 16, 1925)
97. Hickman, T. M., Brit. Pat. 308,389 (May 15, 1929)
97a. Hohenstein, K. A., U. S. Pat. 321,109 (June 30, 1885)
98. Holcomb, H. E., U. S. Pat. 2,030,633 (Feb. 11, 1936)
99. Hornemann, C., *Farbe und Lack*, **1935**, 557
100. Husse, W., *Farbe und Lack*, **1931**, 269
101. Hutin, A., *Rev. chim. ind.*, **26**, 195 (1917)
102. Hutton, H. W., Brit. Pat. 417,948; Abridg. Spec. 1934 Group III, 293
103. Iddings, C., Australia Pat. 20,249 (1934)
104. Iddings, C., U. S. Pat. 2,023,389 (Dec. 3, 1935)
105. Iddings, C., U. S. Pat. 2,047,143 (July 7, 1936)
106. Iddings, C., U. S. Pat. 2,090,630 (Aug. 24, 1937)
107. Iddings, C. and Kennedy, J. N., U. S. Pat. 2,045,551 (June 23, 1936)
108. I. G. Farbenind. A.-G., Brit. Pat. 383,238 (Nov. 10, 1932)
109. I. G. Farbenind. A.-G., Brit. Pat. 396,737 (Aug. 9, 1933)
110. Imperial Chemical Industries, Brit. Pat. 349,464; Abridg. Spec. 1931, Group III, 160
111. Imperial Chemical Industries, Brit. Pat. 447,753; Abridg. Spec. 1936, Group III, 134, Group V, 99
112. Imperial Smelting Corpn., Brit. Pat. 472,001; Abridg. Spec. 1938, Group III, 212
113. Isaacs, M. R., Brit. Pat. 463,725; Abridg. Spec. 1937, Group III, 69
114. Isenberg, D., U. S. Pat. 1,757,864 (May 6, 1930)
115. Jaeger, A., U. S. Pat. 1,830,906 (Nov. 10, 1931)
116. Jones, A., *Paint Manuf. (London)*, **2**, 247, 280, 297 (1932)
117. Jordan, L. A., *J. Soc. Chem. Ind.*, **48**, No. 4, 13T (Jan. 25, 1929)
118. Judd, D. B., *Bur. Standards J. of Research*, **19**, 287 (1937), particularly pp. 302-305 by E. F. Hickson and A. J. Eickhoff
119. Kappelmeier, C. P. A., *Verfkroniek*, **9**, 39 (1936)
120. Keghel, M. de, *Rev. prod. chim.*, **26**, 469, 501 (1923)
121. Kelco Company, Bulletin 71 and advertisement *Amer. Paint J.*, Feb. 28, 1938, p. 3
122. Korobov, N. N., U.S.S.R. Pat. 48,941; *Chem. Zentr.*, **108 ii**, 145 (1937)
123. Kraemer and van Elsberg G.m.b.H., German Pat. 537,234; *Chim. ind.*. **27**, 1398 (1932)
124. Krumbhaar, W., *Drugs, Oils and Paints*, **48**, No. 4, 146, 192 (1933); *Official Digest, Paint and Varnish Production Clubs*, No. 140, 312 (1934)
125. Kühl, H., *Farben-Ztg.*, **30**, 2796, 2865 (1925)
126. Kunze, E., *Farbe und Lack*, **1933**, 581
127. Kunze, E., *Farbe und Lack*, **1936**, 569
128. Kunze, O., German Pat. 490,549 (Oct. 16, 1926)
129. Larson, L. H., U. S. Pat. 1,781,019 (Nov. 11, 1930)
130. Lloyd, H. E., U. S. Pat. 1,833,612 (Nov. 24, 1931)
131. Luckiesh, M. and Holladay, L. L., *J. Franklin Inst.*, **212**, 272 (1931)
132. Ludwig, W., *Farben-Ztg.*, **40**, 347 (1935)
133. Ludwig, W., *Farbe und Lack*, **1935**, 389, 399
134. Ludwig, W., *Farbe und Lack*, **1936**, 207
135. Lyons and Co., Brit. Pat. 392,246; Abridg. Spec. 1933, Group III, 185
136. Maclaurin, J. D., Brit. Pat. 373,215; Abridg. Spec. 1932, Group III. 241
137. Magill, F. la F. and Dangelmajer, C., U. S. Pat. 2,104,264 (Jan. 4, 1938); Brit. Pat. 469,754; Abridg. Spec. 1937, Group III, 175; 470,090; Abridg. Spec. 1937, Group IV, 324, Group III, 180

138. Mann, A., *Farbe und Lack,* **1933,** 417, 429
139. Margival, F., *Peintures, pigments, vernis,* **5,** 673 (1928)
140. Marlier, A., Belg. Pat. 416,958 (Sept. 30, 1936)
141. Mellanoff, I. S., U. S. Pat. 1,857,691 (1933)
142. Merz and Wagner, F., *Farbe und Lack,* **1932,** 535
143. Metrolian, le, French Pat. 803,379 (Sept. 29, 1936)
144. Michel, H. M., U. S. Pat. 2,078,504 (Apr. 27, 1937)
145. Millar, C. J., Brit. Pat. 271,149 (Feb. 18, 1926)
146. Montorsi, E., *Vernici,* **12,** 443, 491 (1936)
147. Moross, W. P. D., U. S. Pat. 1,978,141 (Oct. 23, 1934); *Chim. ind.,* **33,** 431 (1935)
148. Mushkatblat, M. M., Bruskin, I. M., and Bespalko, *Za Lakokrasochnuyu Ind.,* **1934,** No. 1, 31; *Chimie et industrie,* **33,** 691 (1934)
149. National Lime Assoc., Bulletin 304C, Whitewash and Cold Water Paints
150. National Paper Process Co., Brit. Pat. 377,245; Abridg. Spec. 1932, Group III, 394
151. New Zealand Coöperative Dairy Co., Ltd., Brit. Pat. 187,200 (Apr. 28, 1922)
152. Nickowitz, M. N., U. S. Pat. 1,746,162 (Feb. 4, 1930)
153. O'Brien, W. J., U. S. Pat. 2,068,066 (Jan. 19, 1937)
154. Ohl, P., *Fachblatt für Maler,* **1936,** No. 5, 91; *Farben-Ztg.,* **7,** No. 6, 212 (1936)
155. Ott, K. and Breyer, B., German Pat. 599,211 (June 27, 1934)
156. Paindavoine, L. and Vassal, C., French Pat. 803,992 (Oct. 13, 1936)
157. Pamfilov, A. V. and Roslyakova, E. N., *J. Applied Chem. (U.S.S.R.),* **9,** 1664 (1936); *Chem. Abst.,* **31,** 3170
158. Pfanner, H., *Z. angew. Chem.,* **48,** 491 (1935)
159. Pfund, A. H., *J. Franklin Inst.,* **191,** 517 (1921)
160. Pohl, R. H., U. S. Pat. 2,085,602 (June 29, 1937)
161. Rankin, D. A. and Uhler, F. G., U. S. Pat. 2,030,226 (Feb. 11, 1936); Australia Pat. 11,679 and 11,680 (1933); Canadian Pat. 372,620 (Mar. 22, 1938)
162. Rasquin, H., *Farben-Ztg.,* **35,** 2229 (1930)
163. Rasser, E. O., *Kunststoffe,* **17,** 197 (1927)
164. Rasser, E. O., *Tech. Mitt. Malerei,* **53,** No. 19, 193 (1937)
165. Reardon Company, *National Painters' Mag.,* 6 (Sept. 1936)
166. Regnier, A. E., U. S. Pat. 1,506,081 (Aug. 26, 1924)
167. Riabov, A. S., U.S.S.R. Pat. 31,532; *Chim. ind.,* **33,** No. 2, 431 (1935)
168. Richards, C. W. and Dodd, H., Brit. Pat. 414,072; Abridg. Spec. 1934, Group III, 238
169. Richter, W., Czech. Pat. 53,436; *Chem. Zentr.,* **108 i,** 1566 (1937)
170. Riddet, W., Valentine, G. M. and McDowall, F. H., *New Zealand J. Sci. Tech.,* **15,** 309 (1934)
171. Roos, C. K., Offutt, J. S., and Scholz, H. A., U. S. Pat. 2,046,296 (June 30, 1936)
172. Scheiber, J., *Farbe und Lack,* **1929,** 68, 80
173. Scheifele and Wagner, H., *Farben-Ztg.,* **40,** 104 (1935)
174. Scherer, R., "Casein, Its Preparation and Technical Utilization," 3d. ed., revised by H. B. Stocks, New York, D. van Nostrand Co., Inc.
175. Scholz, H. A., Canadian Pat. 353,123 (Sept. 17, 1935)
176. Scholz, H. A., U. S. Pat. 1,947,497 (Feb. 20, 1934); 1,947,498 (Feb. 20, 1934)
177. Schorger, A. W., U. S. Pat. 1,808,865 (June 9, 1931)
178. Schwartz, F. F., Brit. Pat. 405,381 (Feb. 8, 1934)
179. Schwartz, F. F., Brit. Pat. 426,154 (Mar. 28, 1935); U. S. Pat. 2,093,487; 2,093,488 (Sept. 21, 1937); French Pat. 768,329 (Aug. 3, 1934)
180. Schwartz, C., U. S. Pat. 2,064,387 (Dec. 15, 1936)

181. Schwarz, R., *Farbe und Lack,* **1933,** 52, 69
182. Schwarz, R., *Farbe und Lack,* **1935,** 388
183. Schweitzer, H., *Farben-Ztg.,* **40,** 239 (1935)
184. Scofield, F., *Natl. Paint Varnish and Lacquer Assoc., Sci. Sect. Circ.,* **552** (Apr. 1938)
185. Searle, A. B., *Paint Manuf.,* **212,** 276, 288 (1934)
186. Shen, Tze-Hui and Sun, Wei, *Chiao-Tung Univ. Research Inst. Ann. Rept. Bur. Chem.,* **3,** 52 (1936) ; *Chem. Abstr.,* **31,** 3303
187. Slikker, D. and Motman, G. W. C. van, Holland Pat. 26,988 (Jan. 16, 1933)
188. Soc. Italiana Pirelli, Brit. Pat. 472,738; Abridg. Spec. 1938, Group III, 226
189. Soc. Nouvelles des Mines de Saint-Champ, French Pat. 748,628 (July 6, 1933)
190. Soc. Ténéo (S. à. r. l.), French Pat. 804,002 (Oct. 14, 1936)
191. Soc. des Vernis Tab., French Pat. 647,161 (1929) ; *Chem. Abst.,* 23, 2584
192. Stericker, W., *Can. Chem. Met.,* **15,** 135 (1931)
193. Stern, E., *Farbe und Lack,* **1933,** 282, 293, 304
194. Stern, E., Brit. Pat. 455,538; Abridg. Spec. 1936, Group III, 265
195. Stevens, H. P., Bearn, J. G. and Stokes, S. C., Brit. Pat. 407,038 (Feb. 28, 1934)
196. Stevens, H. P. and Heaton, N., *Bull. Rubber Growers' Assoc.,* **15,** 600 (1933) ; *Paint and Varnish Prod. Mgr.,* **14,** 12 (Feb. 1936) ; *J. Oil and Colour Chem. Assoc.,* **17,** 8 (1934)
197. Stroms, H., German Pat. 605,484 (Nov. 12, 1934)
198. Swan, J. H., U. S. Pat. 2,025,788 (Dec. 31, 1935)
199. Swartz, M. D., *Paint Oil and Chem. Rev.,* 10 (Feb. 9, 1928)
200. Swartz, M. D., private communication, but quoted also in ref. 86
201. Syndicat d'Etudes et de Realisations Industrielles et Financieres, French Pat. 729,484 (Mar. 16, 1931)
202. Tarasov, K. and Zitler, G., *Izvestiya Tekstil. Prom Torgov.,* **10,** No. 12, 55 (1931) ; *Chimie et industrie,* **28,** 397
203. Tröger, G., *Farbe und Lack,* **1933,** 472, 487, 499, 516
204. Trumbull, H. L. and Dickson, J. B., U. S. Pat. 1,513,139 (Oct. 28, 1924)
205. Vanderbilt Company, Brit. Pat. 448,458; Abridg. Spec. 1936, Group III, 147
206. Vogel, C., German Pat. 645,619; *Seifen-Ztg.,* **64,** 560 (1937)
207. Wagner, F., *Farbe und Luck,* **1928,** 20, 30
208. Wagner, F., *Farbe und Lack,* **1929,** 187, 204
209. Wagner, F., *Farbe und Lack,* **1930,** 358, 374, 384
210. Wagner, H., *Farben-Ztg.,* **29,** 1503 (1924) ; *Chem. Ztg.,* **48,** 793 (1924) ; **60,** 393 (1936) ; *Progress Interpreted,* No. 44 (June 1936) ; *Kolloid-Z.,* **77,** 12 (1936)
211. Wagner, H., *T R A-Nachrichten,* **1936,** No. 11, 5; *Farben-Chem.,* **7,** No. 4, 137 (1936)
212. Wagner, H. and Kesselring, J., *Farben-Ztg.,* **34,** 947 (1929)
213. Walter, P., *Recherches et inventions,* **16,** 276 (1935)
214. Wiley, W. J., *J. of the Council for Sci. and Indus. Research (Australia),* **7,** 11 105 (1932)
215. Williams, G. M., *J. Soc. Dyers and Colourists,* **43,** 296 (1927)
216. Wilson, W. C., *Paint Oil and Chem. Rev.,* **95,** 14 (1933)
217. Wolff, H. and Zeidler, G., *Farben-Ztg.,* **42,** 357, 383 (1937)
218. Work, L. T. and Odell, I. H., *Ind. Eng. Chem.,* **25,** 411 (1933)
219. Wulf, H., *Farbe und Lack,* **1936,** 171
220. Wulfing, J. A. v., and Busch, A., Brit. Pat. 284,450 (Jan. 11, 1927)
221. Yoshida, S., Brit. Pat. 461,109; Abridg. Spec. 1937, Group III, 19
222. Zatocil, F., Czech. Pat. 52,548; *Chem. Zentr.,* **108 i,** 1566 (1937)
223. Zerr, G., *Farben-Ztg.,* **38,** 1289, 1314, 1338, 1360, 1469 (1933)

Chapter 11

Casein in the Leather Industry

EDWIN S. CAVETT

The use of casein in the leather industry is confined almost entirely to the last of the finishing operations, which consist in coating leather with certain preparations and then subjecting it to mechanical operations such as glazing, plating, brushing, and ironing. After finishing in this way the leather is said to have been seasoned. The preparations applied to the leather are called seasoning materials unless they contain pigments, in which case they are called pigment finishes. Practically all types of leather are finished at least to a certain degree with seasoning materials or pigment finishes.[18, 21, 22, 24]

Among the materials widely used for seasonings and binders for pigment finishes are casein, shellac, carnauba and other waxes, egg and blood albumin, Irish moss and other water-soluble gums, rubber latex, and gelatin.[1, 7, 12, 23] Casein is usually used in admixture with other substances, rarely by itself, because casein alone does not possess all the properties necessary for giving the final characteristics and appearance to leather. Sometimes, in fact, casein is only a minor ingredient of the seasoning, acting, for example, as an emulsifying agent.[10, 15, 17, 20] The seasonings and pigment finishes containing casein are aqueous solutions, dispersions, or emulsions; but not all finishing materials used on leather are aqueous. Patent leather, for example, is finished with a linseed-oil varnish containing pigments, and many novelty effects are obtained on embossed and fancy leathers with pyroxylin lacquers[6, 17] or ethyl cellulose lacquers.[3] Casein pigment finishes, however, may be used as base coats under pigment finishes[4, 10] or as printing colors over lacquer base coats[5] to obtain unusual effects.

Seasonings and pigment finishes are applied to leather to make it generally more serviceable and more pleasing to the eye, to conceal imperfections, and to give various color effects. The highly lustrous effects, for instance, seen on glazed kid leather are produced by the proper use of these materials. Leather has now become so widely used in clothing that a great variety of colors and finishes, obtainable only through the use of seasonings and pigment finishes, is necessary to keep pace with changing styles. Another function of leather finishing is to provide resistance to water. In some cases, such as shoe uppers, leather must

be protected against unduly rapid absorption of moisture from the finished side without making the feet uncomfortable by offering too much resistance to the escape of perspiration from the inside. For such purposes aqueous finishes like those made from casein have the advantage over lacquer finishes.[17, 25]

The ability to select properly combinations of seasonings and pigment finishes and to apply them according to the results desired requires great skill and experience, and is really much more of an art than a science. Many scientific findings, however, relative to the making of leather and to the uses of casein have provided the technologist with material for more intelligent handling.

Kind of Casein Used

The leather trade has long considered Argentine casein very satisfactory and indicates a preference for lactic casein probably because the Argentine casein is made in that way. This preference has not been based on scientific comparisons, but rather it arose in the days when it was difficult to purchase domestic casein of uniform properties. There is now very little choice between domestic and imported casein either from the point of view of quality or that of price. Studies of the film characteristics reveal no practical differences, and even if there were slight advantages one way or the other they probably would be undiscernible in the finished product because casein is usually only one constituent of several in the mixture.

The most important requirement of casein is reasonably uniform viscosity of the solutions made from it. Although the viscosity can be adjusted within certain limits by varying the procedure followed in bringing the casein into solution, it is much more convenient to purchase casein of uniform properties so that changes in formula will be unnecessary. Since leather seasonings are applied in relatively viscous condition, caseins of medium ash content and viscosity actually should be more desirable than those of low ash content provided that they are of good solubility. Hevesi[13] specifies that the casein should be completely soluble in 1 per cent ammonium hydroxide, forming a solution with viscosity greater than 4 Engler that does not decompose within 48 hours. He also limits the "acid number" to 10 and the fat content to 0.8 per cent.

Seasonings

Making the Casein Solution

Casein may be dissolved by means of alkalies such as ammonium hydroxide, borax, sodium hydroxide, sodium carbonate, trisodium phosphate, or triethanolamine. Borax and ammonium hydroxide used either alone or preferably together, are most commonly employed. By varying the proportion of alkali and the temperature it is possible to prepare

solutions of high or low viscosity. Generally speaking, using a given combination of borax and ammonium hydroxide, the viscosity is lower the higher the temperature used in dissolving the casein; on the other hand the viscosity increases in proportion to the amount of borax used at any given temperature up to roughly 80°C. (174°F.). Above this temperature, and especially if the solution is boiled, the viscosity is lower. Ammonium hydroxide produces solutions of lower viscosity than does borax. Borax solutions are more stable and show less change over a period of several days. Solutions in ammonium hydroxide, however, are said to make more water-resistant finishes because they "contain no neutral salts or excess of alkali," but there appears to be no experimental background for these statements and they might well be reëxamined.

There are many variations of the relatively simple process of dissolving casein, but apparently, regardless of these differences, the resulting solutions satisfy the users. A procedure that has merit and gives a solution entirely satisfactory for practically all leather uses is as follows: soak 100 pounds of casein of 70-mesh fineness in 50 gallons of cold water, preferably over night. In a separate container place 37.5 pounds of borax in about 20 gallons of water and heat to boiling to dissolve it. Bring the temperature of the casein suspension to roughly 50°C. (122°F.) and then add the borax solution all at once Stir continuously for at least 15 minutes; gradually raise the temperature to approximately 70°C. (158°F.) in about one hour, stirring at intervals. Then add 4 pounds of 28 per cent ammonium hydroxide and keep the temperature at 70°C., or raise it to boiling and boil if desired. Next add enough water to bring the total volume to 100 gallons and permit the solution to cool. It is advisable to stir the solution occasionally during the cooling period. When cool add as a preservative 2 pounds of parachlormetacresol dissolved in warm water. This procedure gives a solution of moderately high viscosity that serves as a stock solution, the viscosity and concentration being higher than is generally desired for the seasonings. The direction to boil the solution may seem startling to those not familiar with the leather industry. This procedure may not be appropriate for purposes other than leather finishing, but some leather finishers, believe that it gives better solutions. There is, however, difference of opinion within the industry on the subject. Advantages claimed for boiling are that the solutions remain undecomposed longer, and are less subject to changes in viscosity on standing, which makes it possible to prepare successive solutions more uniformly. Others maintain that similar stability is attainable without boiling by making less alkaline solutions and by adding preservatives.[27]

Casein also dissolves and forms a "smooth" solution in dilute hydrochloric acid. The maximum amount dissolves at approximately pH 2.18. The solution may be prepared as follows: soak 48 pounds of casein over

night in 200 pounds of water. Next morning make a dilute solution of HCl by dissolving 9.5 pounds of concentrated hydrochloric acid in 100 pounds of water. Add one-half of the acid solution to 400 pounds of boiling water. Then add the casein suspension slowly with stirring and continued boiling. Finally add gradually the other half of the acid solution and continue to boil until a smooth solution results. This solution makes a clear, bright finish for naturally finished vegetable tanned leather, commonly known as russet leather.

Application of Seasoning to Leather

Seasoning materials are usually applied to leather by hand by means of a swab, constructed by covering a thin board 4 inches wide, 12 inches long and one-half inch thick with plush and having a strap across the back under which the hand is slipped. Seasoning machines are also in general use. From one to five coats of the seasonings are applied, each coat being allowed to dry before the next is applied. After one coat is applied, the leather is hung up to facilitate quick drying and usually the next coat may be applied within an hour, depending upon drying conditions in the tannery.

A typical example of the simplest use of these materials is the seasoning of a naturally finished sheepskin russet leather on the grain or hair side to produce a brilliant luster. For this purpose egg albumin alone would suffice because it develops the highest luster of all the seasoning materials. Casein could also be used alone but the degree of luster obtained would not meet the requirements. A combination of the two is superior to either alone because the casein strengthens the film, increases the binding power, and cheapens the mixture without detracting from the properties of the egg albumin. The concentration of the seasoning materials varies usually from one-tenth to one-half pound of total solids per gallon, depending on the results desired and the kind of materials used. In this case a mixture of one part casein and two parts egg albumin of a concentration of approximately one-fourth pound of total solids per gallon gives good results. Two thin coats are better than one heavy coat. This application, after drying, shows a decided luster but if a brilliant luster is desired the leather must be glazed.

Glazing is necessary if the best results are to be obtained from the seasonings. Much skill and knowledge are required to select the optimum combination of materials. The operation is performed by a machine so constructed as to cause a solid glass cylinder 5 inches long by 2.5 inches in diameter, held fixed in a jaw with axis horizontal, to move rapidly across the surface of the leather under high pressure. The heat generated by the friction causes the seasoning to take on a high luster. The degree of luster can be regulated by varying the pressure of the glass on the leather, the higher the pressure the higher the luster.

solutions of high or low viscosity. Generally speaking, using a given combination of borax and ammonium hydroxide, the viscosity is lower the higher the temperature used in dissolving the casein; on the other hand the viscosity increases in proportion to the amount of borax used at any given temperature up to roughly 80°C. (174°F.). Above this temperature, and especially if the solution is boiled, the viscosity is lower. Ammonium hydroxide produces solutions of lower viscosity than does borax. Borax solutions are more stable and show less change over a period of several days. Solutions in ammonium hydroxide, however, are said to make more water-resistant finishes because they "contain no neutral salts or excess of alkali," but there appears to be no experimental background for these statements and they might well be reëxamined.

There are many variations of the relatively simple process of dissolving casein, but apparently, regardless of these differences, the resulting solutions satisfy the users. A procedure that has merit and gives a solution entirely satisfactory for practically all leather uses is as follows: soak 100 pounds of casein of 70-mesh fineness in 50 gallons of cold water, preferably over night. In a separate container place 37.5 pounds of borax in about 20 gallons of water and heat to boiling to dissolve it. Bring the temperature of the casein suspension to roughly 50°C. (122°F.) and then add the borax solution all at once. Stir continuously for at least 15 minutes; gradually raise the temperature to approximately 70°C. (158°F.) in about one hour, stirring at intervals. Then add 4 pounds of 28 per cent ammonium hydroxide and keep the temperature at 70°C., or raise it to boiling and boil if desired. Next add enough water to bring the total volume to 100 gallons and permit the solution to cool. It is advisable to stir the solution occasionally during the cooling period. When cool add as a preservative 2 pounds of parachlormetacresol dissolved in warm water. This procedure gives a solution of moderately high viscosity that serves as a stock solution, the viscosity and concentration being higher than is generally desired for the seasonings. The direction to boil the solution may seem startling to those not familiar with the leather industry. This procedure may not be appropriate for purposes other than leather finishing, but some leather finishers, believe that it gives better solutions. There is, however, difference of opinion within the industry on the subject. Advantages claimed for boiling are that the solutions remain undecomposed longer, and are less subject to changes in viscosity on standing, which makes it possible to prepare successive solutions more uniformly. Others maintain that similar stability is attainable without boiling by making less alkaline solutions and by adding preservatives.[27]

Casein also dissolves and forms a "smooth" solution in dilute hydrochloric acid. The maximum amount dissolves at approximately pH 2.18. The solution may be prepared as follows: soak 48 pounds of casein over

night in 200 pounds of water. Next morning make a dilute solution of HCl by dissolving 9.5 pounds of concentrated hydrochloric acid in 100 pounds of water. Add one-half of the acid solution to 400 pounds of boiling water. Then add the casein suspension slowly with stirring and continued boiling. Finally add gradually the other half of the acid solution and continue to boil until a smooth solution results. This solution makes a clear, bright finish for naturally finished vegetable tanned leather, commonly known as russet leather.

Application of Seasoning to Leather

Seasoning materials are usually applied to leather by hand by means of a swab, constructed by covering a thin board 4 inches wide, 12 inches long and one-half inch thick with plush and having a strap across the back under which the hand is slipped. Seasoning machines are also in general use. From one to five coats of the seasonings are applied, each coat being allowed to dry before the next is applied. After one coat is applied, the leather is hung up to facilitate quick drying and usually the next coat may be applied within an hour, depending upon drying conditions in the tannery.

A typical example of the simplest use of these materials is the seasoning of a naturally finished sheepskin russet leather on the grain or hair side to produce a brilliant luster. For this purpose egg albumin alone would suffice because it develops the highest luster of all the seasoning materials. Casein could also be used alone but the degree of luster obtained would not meet the requirements. A combination of the two is superior to either alone because the casein strengthens the film, increases the binding power, and cheapens the mixture without detracting from the properties of the egg albumin. The concentration of the seasoning materials varies usually from one-tenth to one-half pound of total solids per gallon, depending on the results desired and the kind of materials used. In this case a mixture of one part casein and two parts egg albumin of a concentration of approximately one-fourth pound of total solids per gallon gives good results. Two thin coats are better than one heavy coat. This application, after drying, shows a decided luster but if a brilliant luster is desired the leather must be glazed.

Glazing is necessary if the best results are to be obtained from the seasonings. Much skill and knowledge are required to select the optimum combination of materials. The operation is performed by a machine so constructed as to cause a solid glass cylinder 5 inches long by 2.5 inches in diameter, held fixed in a jaw with axis horizontal, to move rapidly across the surface of the leather under high pressure. The heat generated by the friction causes the seasoning to take on a high luster. The degree of luster can be regulated by varying the pressure of the glass on the leather, the higher the pressure the higher the luster.

Too much pressure, however, tends to burn the seasoning while too little pressure fails to give maximum brilliance. It is a standard practice to glaze nearly all the seasonings and pigment finishes.

A common operation following glazing is smooth plating. The purpose of this operation is to make the surface smooth, to flatten out the leather and make it firmer, and to anchor the seasoning more firmly. Plating is resorted to only when tight, firm leather is desired. It is done by a machine constructed like a toggle press, the leather being placed between two heated and highly polished plates, which compress it under the desired pressure and temperature. Soft leathers like garment and glove leathers, which are finished on the grain side, are ironed instead of plated because plating would destroy the softness characteristic of these leathers. Ironing produces the same effect of smoothing the surface and flattening the leather and is done by hand with an electric iron or by machine. Both plating and ironing enhance the appearance of the leather.

Properties of Casein as a Seasoning Material

The foregoing example demonstrates how casein is commonly used. There are, of course, many possible combinations of these materials to produce nearly any result on any kind of leather. Casein is probably the most important of all the seasonings because of the many purposes to which it lends itself. In a given mixture it increases the body or viscosity of the solution, thus preventing the film from running before it has set. It is a good filler and especially a good binder. It almost always enhances any mixture by increasing its ability to bind or glue itself more tightly to the leather, a very important property in this type of finishing. Owing to the relatively large micellar weight of casein it does not penetrate too deeply into leather and when used in a mixture it acts as a protective colloid in that it prevents other ingredients from being selectively absorbed by the leather. This explains why better films usually are obtained when casein is a part of the mixture.

A great many leathers must withstand a great deal of flexing and stretching. For that reason any film applied to the surface should be as nearly as possible a part thereof and must be just as flexible; but at the same time it must be hard. Casein is hard but it tends to be brittle. This latter property is the chief drawback to its wider use. Casein therefore hardens an otherwise soft mixture, but casein alone does not stand the constant flexing and stretching of the leather without breaking up in many tiny cracks, commonly referred to as egg-shelling. In so doing it fails in accomplishing that for which it was applied. Any effective method of plasticizing casein would be of great value to its wider use in the leather industry. It is quite usual to incorporate oils and glycerol in the casein solution for the purpose of lessening the brittleness.

Some improvement is noted when these materials are used. In the writer's opinion, however, the improved flexibility may be due less to an actual softening of the casein than to formation of a weaker film containing minute imperfections. On the other hand, by the use of these oils and glycerol, casein can be adjusted for use on glove and garment leathers, which must be extremely soft. Besides glycerol some of the substances often used for this purpose are castor oil, sperm oil, pine oil, various sulfonated oils, alizarin oil, and rubber latex.[1, 16, 26] About 8 to 10 per cent of these materials, based on the weight of dry casein, is usually satisfactory.

Casein is an excellent binder for carnauba wax dispersions. Seasonings containing waxes in considerable proportion are not glazed because the temperature of the leather under the glass cylinder is usually higher than the melting point of the wax so that glazing would result in a dull and streaked effect. This type of seasoning is usually brushed to produce the luster. The brushing machine carries a revolving brush and the leather is fed into the machine under sufficient pressure to produce a high polish, the operator allowing it to be drawn into and pulling it out at will until it meets his requirements. Casein materially improves and enhances the use of waxes in seasonings by increasing the binding power, making the film harder, and making the polish more durable.

Top Seasoning

One of the most important uses of casein in leather is in the so-called top seasonings, which are applied for the purpose of making the leather highly water-resistant and for protecting the pigment finish. This important property of casein is used to excellent advantage in the top finishing of hat leather. This leather requires that the finish be highly water-resistant because of its constant contact with perspiration. The water-resistance of casein can be materially improved by treating a casein solution with about 3 per cent of formaldehyde, based on the weight of the dry casein. In this treatment it is necessary that the casein be dissolved by ammonium hydroxide, and the formaldehyde should be added to the hot solution.[8, 10] A more effective method is to hang the recently seasoned leather in an atmosphere of formaldehyde, providing that the seasoning consists largely of casein.[9] A mixture of gelatin and casein is an excellent seasoning for formaldehyde treatment. Leather so seasoned would be rolled instead of glazed, followed by brushing and plating. There is a common practice of treating casein top seasonings for glazing, while they are still fresh, with a dilute formaldehyde solution by spraying in a booth on a drier conveyor, the booth and conveyor acting as a gas chamber in conjunction with the wet surface.[11] Another method of making casein more water-resistant is to treat the solution with aluminum

sulfate, concentrate the solution, dry the residue, grind, and redissolve in ammonium hydroxide.

Sole, belting, bag, case, and strap leathers are largely finished with mixtures of the seasonings like the examples given, except that they are usually rolled under pressure instead of being glazed. The rolling machine is similar to the glazing machine except that, instead of the fixed glass cylinder passing over the leather to generate heat by friction, a metal cylinder is used that is free to rotate. The combination of rolling and brushing produces high luster, but never so high as that obtained by glazing.

Casein is also used in higher concentrations for applying to the flesh side of leather in order to hold down the exposed leather fibers, which are very coarse in comparison with the smooth, tight surface of the grain side. Pigments and chalk are commonly added to such seasoning mixtures for the purpose of giving a uniform color.

Dyes are frequently added to seasonings in order to give them the same color as the leather on which they are to be applied. The dyes should be light-fast, and acid- and alkali-proof.[3, 8]

Pigment Finishes

Pigment finishes, because of the hiding power of the pigments ground in them, have the advantage over the seasonings of concealing defects in the leather[7] and of giving the surface an even, uniform appearance. If the coating is too thick it gives the surface a painted and artificial look[3] that detracts materially from the value of the leather; it also makes a soft leather feel harsh and dry, and gives it the undesirable appearance of oil cloth. The finish properly handled should give maximum protection in the way of a durable film and just enough hiding power to mask uneven tones on the leather and to conceal normal defects. Above all it should not make the leather look or feel unlike leather. The average pigment finish in the hands of a skilled leather finisher accomplishes this task excellently.

Although much of the casein used in the leather industry is consumed in the simple seasoning mixtures just described, considerably more is employed in the manufacture of the pigment finishes and in connection with their use by the tannery. The mixture of seasoning materials used for the vehicle in which the pigments are ground is called the binder. When the resulting pigment finish is later diluted for use on the leather it is usually diluted with solutions and mixtures of the seasoning materials, which are also referred to as binders.

The pigment finish is actually a special type of water paint and many of the details of manufacture are identical with those described in Chapter 10. Unlike seasonings, pigment finishes are not, as a rule, made at the tannery but are purchased from concerns specializing in their

manufacture. The chief difference between pigment finishes and water paints lies in the character of the binder. For leather it must be designed to produce a film that is as flexible as possible without sacrificing hardness, and it must prevent the pigment from lifting or dusting.[2] A water paint is usually put on a fixed, hard surface and therefore does not have to be so flexible.

Formulation of the Binder

Formulating the binder requires skill and experience besides an intimate knowledge of the properties of the seasoning materials.[19, 27]

Stock solutions of casein, shellac, egg and blood albumin, carnauba and beeswax dispersed in soap solution, Irish moss, flaxseed, and gum tragacanth, usually are prepared in a concentration of one pound to the gallon. These stock solutions suffice for any requirement of this type of leather finish. Gelatin is an important material but unless it is given special treatment it cannot be kept in solution in a sufficient concentration. Egg and blood albumin are used to promote high glazing. Shellac is an excellent binding or adhesive material and provides a natural brilliance, but it is brittle and not readily plasticized.[14] Waxes are used for their relative softness and tendency to maintain the "leathery feel" as well as to provide luster when the finish cannot be glazed. Irish moss, flaxseed, and gum tragacanth are good fillers, that is, they tend to penetrate farther into the leather; being softer than some of the others they help make the film more flexible. Flaxseed especially gives the leather a soft feel. Gelatin is an adhesive, a protective colloid, and a good glazing and filling material. Casein in pigment finishes serves in the important capacity of dispersing the pigments, acting as a protective colloid to prevent agglomeration of the pigment particles, and stabilizing the product. Its relatively high viscosity makes it an especially valuable agent in preventing settling of the pigment.

TABLE 29.—Composition of Binder for Hard, Medium, and Soft Pigment Finishes

	Percentage by volume of stock solution		
Stock solution	Hard binder	Medium binder	Soft binder
Casein	40.0	30.0	20.0
Shellac	15.5	30.0	20.0
Carnauba wax dispersed in soap solution	19.5	30.0	40.0
Blood albumin	9.0		
Gelatin	9.0		
Sulfonated castor oil	7.0	10.0	10.0
Irish moss			10.0
	100.0	100.0	100.0

NOTE: The stock solutions contain 1 pound of solute per gallon of water except that the gelatin solution contains 5 pounds of gelatin in 9 gallons of water. The gelatin solution is added while hot.

The binder can be made hard or soft by varying the relative proportions of casein and waxes. There are, of course, many possible mixtures that may be used for the binder but for the sake of illustration the types shown in Table 29 amply meet the ordinary requirements in the hands of the skilled leather finisher. By hard binder is meant one that produces a finish having a hard surface.

Pigments

The selection of the pigments is important, primary requisites being high opacity, chemical inactivity, resistance to acids and alkalies, small particle size, freedom from abrasive material and water-soluble matter, and fastness to light.[1, 6, 14, 19] These requirements are essentially the same as for casein paints, discussed in Chapter 10. The pigment manufacturers have excellent products available for this work; but much skill and experience, together with an appreciation of the chemical natures of both the pigments and the leather, is necessary to select them intelligently.

In general the oxide pigments are satisfactory. For whites, titanium dioxide and combinations thereof, as well as the high-strength lithopones, are best. For reds the iron oxides meet most of the demands for shading and mixing. For brilliant reds the toners must be used; those of the toluidine type are preferable because the nitraniline toners are apt to bleed. Lead chromate yellows and oranges must be used carefully because of their sensitivity to acids and alkalies. Cadmium pigments are very satisfactory but they are expensive and less opaque. The iron oxide yellows and earth colors are also suitable. The iron blues are sensitive to alkalies and must therefore be used very carefully, but the new copper phthalocyanin blues are fast to alkalies. For blacks the iron oxide blacks are best, but carbon black is more opaque. Both have their place. There are many other pigments used for special purposes.

It is customary for the manufacturer of pigment finishes to make stock finishes of the primary colors and then to blend them to produce the desired colors, tints, and shades. When any special color is to be made in large volume, however, it saves time to use a dry color formula and to grind this color rather than to mix the primary stock colors each time.

Grinding the Pigment

Different manufacturers have different procedures for grinding, some preferring to grind the pigment in the oil and wax mixture and then stir in the remaining ingredients. The best practice, however, is to grind the pigment in the completed binder, such as one of those listed in Table 29. Usually three quarts of the binder suffice for one gallon

of finish, the volume of the pigments being enough to make up the balance and to allow for the loss in grinding.

The amount of pigment used per gallon varies from 1 pound to roughly 2.5 pounds for the colors, and from 2 to 4 pounds for the whites. The type of machine most satisfactory for grinding is the pebble or ball mill. Colloid mills and other types probably have their place but for general application the pebble mill is best. For whites and very light colors it is safest to use a flint-lined mill and flint pebbles, whereas for colors steel mills and steel balls are satisfactory. The usual procedure is to charge the mill with the binder to the extent of one-half or three-quarters of the final volume desired, followed by all the pigment, after which the door is put on and the mill rotated for about 16 hours. The charge is then removed and the mill washed with water. The batch is then made up to the desired volume with water or binder according to the individual requirements. This product represents the stock pigment finish as the tanner receives it from the manufacturer of the pigment finish.

Application to Leather

The tanner does not apply pigment finish to leather in the form in which the finish is received; instead he dilutes it to suit his requirements. Knowing the character of the finish required for the leather, he can develop the properties necessary to obtain the desired result by properly choosing the diluting binder mixture and the corresponding mechanical technic. A few examples will best serve to demonstrate this and to show how a given finish can be used to produce different results. Typical examples are the finishing of sheepskin lining leather, used for lining the inside of shoes, and cowhide side upper leather used for making the upper part of men's shoes. Both require a hard finish, and therefore the pigment finish is made with a binder such as the hard binder shown in Table 29. The two finishes as used on the leather are compared in Table 30.

TABLE 30.—Two Examples of Pigment Finishes as Diluted for Use

Stock material		For sheepskin lining leather	For cowhide shoe leather
Stock pigment finish,	gallons.......	25.0	20.0
Water,	gallons.......	51.0	65.0
Casein solution,	gallons.......	14.0	5.0
Gelatin,	pounds.......	4.0	1.0
Flaxseed suspension,	gallons.	5.0	
Shellac solution,	gallons.......		10.0

NOTE: The gelatin is dissolved in the water.

In both cases two coats of the finish are applied, with time for drying between coats. These two coats are sufficient for the sheepskin, but

the cowhide is given a third coat of a dilute seasoning mixture consisting of 12.5 gallons of shellac solution, 12.5 gallons of casein solution and one pound of gelatin dissolved in 75 gallons of water. A hard finish is produced in both cases. The sheepskin is more difficult to cover than the cowhide; therefore more pigment is required. Being on the inside of the shoe it must be capable of resisting the dampness of the feet as well as the constant rubbing of its surface by the feet. It does not have to stand the severe flexing that the cowhide does, which permits the use of a greater proportion of casein. The cowhide must stand up under the ordinary wear of the upper part of the shoe. The principal ingredient in the diluting binder for this leather is shellac, chosen because of its more lasting brilliance and for its adhesive properties. The top seasoning on the cowhide protects the coating of pigment finish against scuffing, weather, and wear, and increases the period of good appearance of the shoe.

Similar examples could be mentioned for other classes of finishes for the different leathers, but the general procedure is the same. In practically every case casein is an important ingredient. There are some cases where pigment finishes are so well balanced in their make-up that they can be merely diluted with water.

Other Uses of Casein

From time to time various other uses for casein in the leather industry are suggested. Casein is sometimes used for clarifying vegetable tanning solutions, as an ingredient of the paste for pasting leather, as a filler for loose fibered, heavy leather, applied by tumbling the leather in a casein solution in a wheel, as a waterproof glue for use in covering machine rolls where wet leather is used, and other uses that are probably of remote interest to the tanner.

References

1. Anonymous, *Paint and Colour Record,* **1,** No. 1, 13 (1931)
2. Anonymous, *Collegium,* **1934,** 196; *Farbe und Lack,* **1935,** 220
3. Artus, F. v., *Farben-Chem.,* **8,** 385 (1937)
4. Blatz, P. A., U. S. Pat. 1,985,236 (Dec. 25, 1934)
5. Blatz, P. A., U. S. Pat. 1,985,675 (Dec. 25, 1934)
6. Boxler, R., *Farben-Chem.,* **6,** 18 (1935)
7. Bryson, H. C., *Paint Manufacturer,* **3,** 69, 97 (1933)
8. Freiman, A. E., *Kozhevenno-Obuvnaya Prom., U.S.S.R.,* **15,** No. 7, 45 (1936); *Chem. Abstr.,* **31,** 5207
9. Galdobin, F. T., *Kozhevenno-Obuvnaya Prom., U.S.S.R.,* **15,** No. 7, 48 (1936); *Chem. Abstr.,* **31,** 5207
10. Galimberti, G. and Peverelli, G., Italian Pat. 314,114; *Farben-Chem.,* **8,** 277 (1937)
11. Geister, C. H., private communication
12. Genin, G., *Halle aux cuirs,* **1928,** 373; **1929,** 13
13. Hevesi, A., *Gelatine Leim Klebstoffe,* **3,** 43 (1935)

14. Hevesi, A., *Farben-Chem.*, **6**, 290 (1935)
15. I. G. Farbenindustrie A.-G., British Pat. 373,233; Abridg. Spec. 1932, Group IV, 312
16. I. G. Farbenindustrie A.-G., German Pat. 652,082; *Seifen-Ztg.*, **64**, 893 (1937)
17. Khomutov, A. M. and Plotnikov, I. V., *Kozhevenno-Obuvnaya Prom., U.S.S.R.*, **15**, No. 12, 62 (1936); *Chem. Abstr.*, **31**, 5186
18. Lamb, M. C., "Leather Dressing," London, Leather Trades Pub. Co., 1909
19. Martin-Wood, H., *Cuir. tech.*, **20**, 224 (1931); *Chem. Abstr.*, **26**, 607
20. Oakeshott, S. H., Stewart, A., and Todd, W., British Pat. 410,503 (May 14, 1934); *Chem. Abstr.*, **28**, 6344
21. Proctor, H. R., "Principles of Leather Manufacture," New York, D. van Nostrand Co., Inc., 1922
22. Rogers, A., "Practical Tanning," New York, Henry Carey Baird & Co., 1922
23. Wieber, O., *Farbe und Lack*, **1932**, 581, 595
24. Wilson, J. A., "The Chemistry of Leather Manufacture," ch. 16, New York, Reinhold Publishing Corp., 1923
25. Wilson, J. A. and Lines, G. O., *Ind. Eng. Chem.*, **17**, 570 (1925)
26. Zeldin, S. P. and Ponomarenko, R., *Prom. org. Khim.*, **3**, 224, 416 (1937); *Chem. Abstr.*, **31**, 6920
27. Zeldin, S. P., *J. Khim. Promychl.*, **8**, No. 15-16, 58 (1931); *Chem. Ind.*, **28**, 401 (1932)

the cowhide is given a third coat of a dilute seasoning mixture consisting of 12.5 gallons of shellac solution, 12.5 gallons of casein solution, and one pound of gelatin dissolved in 75 gallons of water. A hard finish is produced in both cases. The sheepskin is more difficult to cover than the cowhide; therefore more pigment is required. Being on the inside of the shoe it must be capable of resisting the dampness of the feet as well as the constant rubbing of its surface by the feet. It does not have to stand the severe flexing that the cowhide does, which permits the use of a greater proportion of casein. The cowhide must stand up under the ordinary wear of the upper part of the shoe. The principal ingredient in the diluting binder for this leather is shellac, chosen because of its more lasting brilliance and for its adhesive properties. The top seasoning on the cowhide protects the coating of pigment finish against scuffing, weather, and wear, and increases the period of good appearance of the shoe.

Similar examples could be mentioned for other classes of finishes for the different leathers, but the general procedure is the same. In practically every case casein is an important ingredient. There are some cases where pigment finishes are so well balanced in their make-up that they can be merely diluted with water.

Other Uses of Casein

From time to time various other uses for casein in the leather industry are suggested. Casein is sometimes used for clarifying vegetable tanning solutions, as an ingredient of the paste for pasting leather, as a filler for loose fibered, heavy leather, applied by tumbling the leather in a casein solution in a wheel, as a waterproof glue for use in covering machine rolls where wet leather is used, and other uses that are probably of remote interest to the tanner.

References

1. Anonymous, *Paint and Colour Record,* **1,** No. 1, 13 (1931)
2. Anonymous, *Collegium,* **1934,** 196; *Farbe und Lack,* **1935,** 220
3. Artus, F. v., *Farben-Chem.,* **8,** 385 (1937)
4. Blatz, P. A., U. S. Pat. 1,985,236 (Dec. 25, 1934)
5. Blatz, P. A., U. S. Pat. 1,985,675 (Dec. 25, 1934)
6. Boxler, R., *Farben-Chem.,* **6,** 18 (1935)
7. Bryson, H. C., *Paint Manufacturer,* **3,** 69, 97 (1933)
8. Freiman, A. E., *Kozhevenno-Obuvnaya Prom., U.S.S.R.,* **15,** No. 7, 45 (1936); *Chem. Abstr.,* **31,** 5207
9. Galdobin, F. T., *Kozhevenno-Obuvnaya Prom., U.S.S.R.,* **15,** No. 7, 48 (1936); *Chem. Abstr.,* **31,** 5207
10. Galimberti, G. and Peverelli, G., Italian Pat. 314,114; *Farben-Chem.,* **8,** 277 (1937)
11. Geister, C. H., private communication
12. Genin, G., *Halle aux cuirs,* **1928,** 373; **1929,** 13
13. Hevesi, A., *Gelatine Leim Klebstoffe,* **3,** 43 (1935)

14. Hevesi, A., *Farben-Chem.*, **6**, 290 (1935)
15. I. G. Farbenindustrie A.-G., British Pat. 373,233; Abridg. Spec. 1932, Group IV, 312
16. I. G. Farbenindustrie A.-G., German Pat. 652,082; *Seifen-Ztg.*, **64**, 893 (1937)
17. Khomutov, A. M. and Plotnikov, I. V., *Kozhevenno-Obuvnaya Prom., U.S.S.R.*, **15**, No. 12, 62 (1936) ; *Chem. Abstr.*, **31**, 5186
18. Lamb, M. C., "Leather Dressing," London, Leather Trades Pub. Co., 1909
19. Martin-Wood, H., *Cuir. tech.*, **20**, 224 (1931) ; *Chem. Abstr.*, **26**, 607
20. Oakeshott, S. H., Stewart, A., and Todd, W., British Pat. 410,503 (May 14, 1934) ; *Chem. Abstr.*, **28**, 6344
21. Proctor, H. R., "Principles of Leather Manufacture," New York, D. van Nostrand Co., Inc., 1922
22. Rogers, A., "Practical Tanning," New York, Henry Carey Baird & Co., 1922
23. Wieber, O., *Farbe und Lack*, **1932**, 581, 595
24. Wilson, J. A., "The Chemistry of Leather Manufacture," ch. 16, New York, Reinhold Publishing Corp., 1923
25. Wilson, J. A. and Lines, G. O., *Ind. Eng. Chem.*, **17**, 570 (1925)
26. Zeldin, S. P. and Ponomarenko, R., *Prom. org. Khim.*, **3**, 224, 416 (1937) ; *Chem. Abstr.*, **31**, 6920
27. Zeldin, S. P., *J. Khim. Promychl.*, **8**, No. 15-16, 58 (1931) ; *Chem. Ind.*, **28**, 401 (1932)

Chapter 12

Alimentary and Medicinal Uses of Casein

H. A. Schuette

In the uncombined state in which casein is marketed as a by-product of milk processing operations, it is not valued *per se* as a food product. In its modified condition as cheese, however, it has always had a place in the dietary of all nations, although its value when measured in terms of modern nutrition yardsticks may not have been fully appreciated. It has been found, also, to be well adapted chemically and physically to the manufacture of medicinal foods, or preparations of alimentary substances answering special needs in the nutrition of infants, the sick and the convalescent, and in the dietotherapy of diabetes. To that end it is regarded as a good vehicle for administering such remedial agents as heavy metals, tannins, alkaloids, salicylates and iodides. In combination with these substances casein markedly modifies their physiological properties and thereby enhances their value for medicinal purposes. Acid or alkaline solutions of casein are colloidal. They are good emulsifying agents and act as protective colloids. This important property also is used to advantage in the preparation of therapeutic substances.

From the standpoint of nutritive value, casein compares very favorably with the proteins of meat.[47] In the form of its amino acids, it is readily absorbed in the intestinal canal, metabolism experiments having shown a utilization[6] of about 98.5 per cent. This is more than that obtained with proteins of vegetable origin. Obsorne and Mendel,[54] in a comparison of the biological values of individual proteins when fed at an 18 per cent level with other substances in the diet, demonstrated that casein is superior to all other proteins in promoting growth, and Mitchell[50] found it to be more effective than egg white in increasing body weight.

Casein is referred to as "complete" in that it is built up of amino acids not only of proper kind and amount for growth and adequate nourishment, but of the requisite quality as well. Among its hydrolysis products are at least fifteen amino acids (see Table 2 in Chapter 2), significant among them being tryptophane and glutamic acid. Others are alanine, valine, leucine, phenylalanine, tyrosine, serine, cystine, proline, hydroxyproline, aspartic acid, arginine, lysine and histidine. Una-

nimity of opinion as to the presence of glycine apparently does not exist although the weight of experimental evidence points to its absence.

Besides its amino acids, casein contains also phosphorus, which fact gives rise to its classification as a phosphoprotein. The significance of this element in the growth, development and functional activities of the animal organism has received but tardy recognition, yet it is pertinent to note that fully 90 per cent[48] of the phosphorus of casein is utilized by the body.

The foregoing considerations, it is believed, definitely establish the food value of casein and in them may be found at least a partial explanation of why casein has been used in such a variety of ways for alimentary preparations.

Casein is also prized for its contribution to the diet by way of vitamins. Animal experimentation has demonstrated, however, that when it is precipitated from a solution of its sodium compound and then shaken with Fuller's earth in the presence of hydrogen peroxide, it becomes inadequate in nutritive efficiency.[32] Such a casein is claimed to be vitamin-free.[31]

In combination with alkalies, as in the so-called caseinates, casein is soluble in water, and is therefore readily acted upon by the digestive juices. The caseinates have a satisfactory degree of stability if kept dry, and in this condition are without odor. Experimental evidence shows that from a nutritive standpoint their value is very similar to that of casein itself.[15, 70]

Casein-containing preparations are numerous, for "within recent years," writes Seaber,[65] "there has been a growing demand for food substances which shall take the place of the flesh-building part of meat. This is perhaps largely owing to the increase of vegetarianism and though many hardened meat eaters will look with disgust upon such preparations, yet they are very valuable on account of the nourishment they are capable of affording without overloading the system."

The use of casein or its compounds in foods finds expression in instances of which the following are typical: reconstituted or synthetic foods; foods for children, for the diabetic and the dyspeptic; in infants' foods; in reconstructed, modified, malted and "albuminous" milks; in food adulterants; bakers' wares; as ingredients of so-called egg substitutes; as an ice-cream filler; in soup tablets; as an adjunct in the manufacture of oleomargarine; and in milk chocolate and cocoa.

Commercial casein suitable for the needs of the arts does not possess the requisite degree of fineness, purity, solubility, and digestibility for either pharmaceutical or alimentary purposes, where a product substantially free from color and odor and of a fine texture is necessary. While the requirements may vary in individual cases, casein intended

for use in pharmaceutical preparations should have an ash content below 2 per cent and a minimum fat content of one per cent.

Van Slyke and Baker[78] have described an excellent laboratory method for producing a pure casein free from cleavage products and calcium and remarkably low in inorganic phosphorus. It is obtained by this procedure as a fine, white powder which dissolves quickly to a clear solution in dilute solutions of monobasic alkalies or an excess of lime water. This grade of casein, however, is of a higher quality than that required for the preparation of pharmaceutical compounds. Its large-scale production does not seem practical.

To overcome the difficulty referred to, Clark and associates[17] have developed a method, known as the "grain curd" process, for producing a high-grade casein which can be put into large-scale operation. By this method, which is described in Chapter 4, there is produced a casein which is purer than the ordinary industrial variety and which for most purposes is well adapted to the manufacture of the products in question.

Reconstituted or Synthetic Foods

The knowledge that a liquid closely resembling milk and cream can be made by emulsifying unsalted butter fat in a solution or suspension of skim-milk powder is not new. The ice-cream industry uses this procedure very widely.

From an economic standpoint and in the interests of food conservation, desiccation of milk may sometimes be desirable, if not necessary; and from purely monetary considerations storage and transportation of the community's milk supply on a water-free basis offer attractive possibilities. The ethical aspects of handling reconstructed milk under its own label and with complete information as to source and method of preparation are beside the purpose of this discussion. Skim-milk powder containing casein, sweet or unsalted butter as a source of butter fat, and water, form the ingredients which, when properly mixed, result in a product for which names such as "remade," "emulsified," "reconstructed," "recombined," and "rehydrated" have been suggested. The exigencies of the late war brought such a product into prominence at Nitro, W. Va., under conditions that made possible its study from a commercial standpoint.[69]

A synthetic milk compound and a process of producing it was described some 35 years ago by Hall,[34] who suggested dissolving dry casein in sodium bicarbonate and then adding a small percentage of calcium chloride. To that mixture milk sugar and butter fat were then to be added. The addition of water completed the product.

A stable suspension of casein in water for use as a milk substitute in bread baking was made by Dunham.[26] He dissolved casein in an

alkali, made the solution acid to litmus by the addition of an acid citrate, thus precipitating the casein in a colloidal form, and then dehydrated the mass in a milk-drying machine of appropriate design. A somewhat similar use of a citrate is claimed by Backhaus,[5] who converted casein into an "appetizing food product" by the use of trisodium citrate in the presence of sodium bicarbonate or trisodium phosphate.

From German sources comes information[16] of a casein product that may be boiled with water without the separation of a gelatinous precipitate. This is made by mixing casein or its salts with albumin or peptone-containing substances. Monhaupt's patented preparation, referred to later in this chapter, also belongs in this class.

It may be of more than passing interest to note that König[41] some thirty-five years ago took the position that no pecuniary advantage accrues to agriculture in the utilization of skim milk for the production of patented preparations of the type exemplified by Plasmon, "Kalk-casein," Nutrose, Sanatogen, Eukasin, Galaktagen, Eulaktol, "Milcheiweiss 'Nickol'," and "Sanitätseiweiss 'Nickol'," in view of the fact that the cost of proteins therein contained is for the masses, by and large, greater than those of milk and cheese, the nutritive value of the caseinates in question to the contrary notwithstanding.

Foods for Children

A milk food known as Guttmann's nutrient flour, the chief characteristic of which is a high content of readily assimilable carbohydrates, appeared on the German market some years ago. This is said to be very digestible and especially adapted to the needs of children and of dyspeptics. According to Jolles,[39] its base is skim milk casein to which are added prepared carbohydrates, such as gluten-free oatmeal, and a small proportion of aromatic substances for the purpose of imparting a flavor to the mixture. Carbohydrates make up about 65 per cent of the dry matter, nitrogenous matter is present to the extent of 20 per cent, and the fat content is 4 per cent.

Sell[66] prepared from casein what is designated as an easily digestible food product. At the will of the operator its nutritive value may be enhanced by the addition of lactose or its content of milk solids increased by the addition of a concentrated whey. The action of added kefir "grains" aids in breaking down the whole mass. The acidity of the reaction product is reduced, if not entirely neutralized, and then the mass is absorbed on zwieback or similar dry food, and then dried.

For the preparation of a product with some of the characteristics of a baked food, Liebrecht[45] makes an intimate mixture of casein or its salts with animal or vegetable fats and then heats it to 150° to 200°C.

Foods for Diabetics

It is probably not too much to say that there is no universal food for diabetics; for most foods may be suitable under certain conditions for the use of persons afflicted with the disease. The discovery of newer methods of diabetes therapy has rendered obsolete the definition that food for diabetics contains not more than half as much glycogenic carbohydrates as a normal food of the same class.[76] Although the number of proprietary foods on the market making claim to this doubtful distinction is almost legion, they are being replaced by more satisfactory dietary regimens which, because of the discovery of insulin, are based on the carbohydrate tolerance of the patient.

In a compilation of some 630 foods for diabetics recently made at the Connecticut Agricultural Experiment Station,[7] only five were found in which casein was either known to be present or mentioned on the label. With one exception the five samples in question were casein flours whose protein content ranged from 72 to 79 per cent. The analyses of these preparations are given in Table 31.

A milk for diabetics, said to be sugar-free, of minimum protein but high fat content, and possessing a pleasant taste, is described in the *Milch Zeitung*.[2] Information about its practical value is not available, but it is believed that its sale can be only local. Its composition for a "five per cent" product was found to be: fat 4.96 per cent, protein 1.14 per cent (62.5 per cent of which was casein), mineral matter 0.17 per cent, nitrogen-free substances 1.24 per cent, and water to make 100 per cent. Similarly a 10 per cent milk had an indicated fat content of 9.98 per cent with a protein value of 2.29 per cent.

A product known as Lister's Casein Palmnut Dietetic Flour[19] is proposed for use in the dietetic treatment of diabetes and wherever restriction of carbohydrate is desired. It consists of wheat, edible nuts and casein to which has been added, besides sodium chloride, a leavening mixture composed of potassium acid tartrate and sodium bicarbonate. Its composition is said to be: protein 28.67 per cent, carbohydrates 28.68 per cent, fat 18.69 per cent, mineral matter 5.64 per cent, crude fiber and pentosans 7.59 per cent, moisture 8.49 per cent.

Diaprotein Prepared Casein Flour N. N. R. is another commercial food intended for the diabetic. It consists of casein to which has been added 4 per cent of a leavening mixture composed of sodium bicarbonate, monocalcium phosphate and sodium aluminum sulfate. It is sold in the form of a flour which may be used in the preparation of bread, cake or muffins. In the same category falls another proprietary product known as Caseinhydrol[4] the constituents of which are casein, calcium phosphate and magnesium oxide.

TABLE 31.—Composition of Foods for Diabetics
(*Data of Bailey*[7])

Name of food	Water (%)	Ash (%)	Nitrogen (%)	Protein (nitrogen times 6.25) (%)	Crude fiber (%)	Starch (%)	Other nitrogen-free extract (%)	Fat (%)
Alpha Best Diabetic Wafer	6 81	6 23	7 74	48 38	0 17	....	4 89	33 52
Diabetic Casein Flour	11 93	9 16	11 56	72 25	0.14	..	5.73	0 79
Prepared Casein Diabetic Flour	11 53	9 44	10 93	68 31	0 05	...	9 72	0 95
Diaprotein Casein Flour	11 72	6 35	12 44	77 75	...		2 72	1 46
Snow Flake Diabetic Casein Flour	10 41	6 07	12 67	79 19	..	...	3 18	1.15

Infants' Foods

It is through the medium of powdered skim milk that casein enters into the composition of many proprietary foods intended for infant feeding. Often casein itself is used, being modified to a certain extent by the introduction of substances like the phosphates or precipitated in a way to render it substantially ash-free. In an attempt to obtain a product possessing at least some of the properties of the casein of mothers' milk and more easily digested in the infantile stomach, casein may also be subjected to the action of digestive ferments before it is compounded into infant foods.

Bosworth and Chrysler[13] treat milk with disodium phosphate or other substance containing the phosphate radical under conditions that do not disturb the solution of the casein in its natural condition. The milk is then rendered slightly alkaline in order to precipitate insoluble calcium phosphate, which is then removed. Neutralization, if necessary, follows this operation. The product so obtained is claimed to be suited for the preparation of milk for feeding infants.

In preparing a "reconstructed" milk of approximately the same composition as human milk, Bosworth[11] separates the fat and the casein from the serum of cow's milk and, by the addition of a limited quantity of lime water, removes substantially all the calcium and phosphorus from the serum. The treated serum is then combined with a portion of the fat and with olive oil "and other modifying components" in the requisite proportions. The claim is advanced that the removal of excess calcium from milk results in an almost complete digestibility and assimilability of all the remaining food elements.

An albuminous milk which Neukirch[52] considers an anti-fermentative and a satisfactory substitute for human milk is prepared by diluting buttermilk with an equal volume of water and two volumes of milk

to which casein has been added. The carbohydrate content of the whole is increased by the addition of a dextrin-maltose mixture. The method of preparation followed in the manufacture of Riegel's[60] milk albumin is of interest because the precipitant used is said to yield an almost ash-free casein which is thrown out of solution in a non-mucilaginous condition. Ethylsulfuric, rather than acetic or sulfuric acid, is used to precipitate the curd from milk previously diluted.

Nature intended the infant as well as the calf to obtain its nourishment from milk, but although cow's milk is a perfect food for the calf, it is very imperfectly adapted to the infant. The principal differences are that human digestion is largely intestinal while bovine digestion is stomachic, and that cow's milk in the infant's stomach forms large curds. To overcome this condition the milk must be so treated as to cause the curds to form as minute, soft, flocculent particles. With this in view, Dunham[23] treats casein with a digestive ferment, such as pancreatin, in order partly to peptonize it and at the same time to render it soluble. The curd obtained by acid precipitation is washed free of acid and milk sugar and then dissolved in sodium bicarbonate solution. In this condition it is subjected to the action of pancreatin for twenty to thirty minutes at an elevated temperature. The action is then arrested by drying the product in thin films on cylinders heated to 100°C. After it has been pulverized it is ready for use.

Malted Milk

Malted milk is the product resulting from the combination of whole milk with an extract of malted barley and wheat flour in such a manner as to bring about the full enzymic action of the malt extract. It may also contain sodium chloride, and sodium or potassium bicarbonates. Federal standards require that it contain at least 7.5 per cent milk fat and a maximum of 3.5 per cent of moisture. The whole is reduced to dryness by desiccation. It is the only milk powder made from whole milk that has good keeping qualities and these are ascribed to the protective coating of gluten, sugars and salts which surrounds the fat globules.

Horlick's discovery of the method of manufacturing this food product some forty years ago has made available a preparation that combines a high degree of digestibility and nutritive value with the growth-promoting properties of whole milk. Casein enters into its composition as a normal constituent of the milk from which it is made. A typical method of manufacture follows: A mash of wheat flour and barley malt of good diastatic quality is allowed to age long enough to permit complete conversion of the starch into dextrin and maltose, a process similar to that of digestion in the human system. This extract is then combined with whole milk and the mixture reduced to powdered form

by vacuum evaporation. The temperature used is sufficient to cause pasteurization without affecting the digestibility of the product. Hunziker[38] finds that the total protein content of malted milk varies from 10 to 15 per cent, the casein content lying between 6.4 and 9.25 per cent.

Food Adulterants

It has come to the attention of food law enforcement officials that some dairymen actually work casein in the form of milk powder or cottage cheese into their butter and cream for the purpose of increasing their water-holding capacity. The practice, however, is rather vigorously opposed within the industry itself.[68] Occasional instances of this form of adulteration of Dutch butter have come to light in the past, the presence of casein making itself apparent by the separation of an unusual amount of coagulated matter from the melted butter. An analysis of such a butter is reported by Racine[56] as: casein 5.49 per cent, ash 0.77 per cent, water 24.73 per cent, and fat 69.01 per cent.

At the present time, however, food law enforcement officials of the Department of Agriculture report that if the practice exists at all today it is strictly local in character and affects only an infinitestimal amount of the production. Since the enactment of the Food and Drugs Act in 1906, particular attention has been given to butter, and adulteration with curd has not been found on any appreciable scale.

Cream is another dairy product that has sometimes fallen into similar evil ways. As thickening agents there have come to light preparations consisting of cane sugar and gum tragacanth, and sometimes casein.[11] Revis and Bolton[59] report the fraudulent use of casein and dried and condensed milk for this purpose. The addition of these substances can usually be detected, it is stated, by an unusually high content of solids other than fat. For the detection of casein it is suggested that the suspected cream be diluted with an equal volume of boiling water and that the mixture be strongly centrifuged. If casein has been added, a gummy mass of undissolved casein is usually obtained. Furthermore, cream containing added casein is in most cases frothy on account of the agitation employed to incorporate the thickening agent.

The ability of casein to absorb a large amount of water finds practical application in its incorporation with sausage meat, particularly meat that has become dry and therefore partially lost its binding power. In Germany the materials used to increase the moisture content of sausage meats have been found to be albuminous in nature, consisting of desiccated egg or serum albumin, casein, or gluten. The objection to this practice is obvious because in effect it substitutes water for

meat and greatly lowers the nutritive value of the food per unit weight.

Mansfeld[46] *reports the composition of a preparation the function of which is to serve as a binding material in sausage meat.* Analysis indicated that it contained 65.86 per cent casein and 12.24 per cent ash constituents, the latter being largely sodium bicarbonate. Inasmuch as it exhibited a decided tendency to absorb water, it appears that it was well suited for the purpose for which it was intended.

Another product of this type, appearing during the present decade in Switzerland, has been described by Kreis and Iselin.[42] It, too, is a casein product and apparently is an old one that had been tagged with the new name of "Milpu." Its predominating constituent is reported to be protein 64.7 per cent, lactose 1.3 per cent, ash (largely calcium phosphate) 15 per cent, and moisture 19 per cent.

Bakers' Wares

Casein has no general use in the baking industry in the United States except insofar as it is a constituent of the milk used in making breads. To a limited extent it enters into special products intended for dietary purposes. Dry skim milk has been found to have a beneficial effect upon the volume of the loaf and upon the texture of the bread, when it is incorporated into the dough mix in quantities not exceeding 6 per cent of the weight of the flour used. It is probable that these beneficial effects may be due to some property of the casein.

In the addition of various ingredients it is well to keep in mind the fact that an acid rather than an alkaline reaction is desirable. The former reaction acts as a control upon the fermentation and thereby prevents the development of "rope."

In describing the preparation of an easily digested bread for convalescents, Scherer[61] states that it should possess a light consistency and neutral reaction and be of high protein content. To that end Hahn and Planitzer[33] prepare a wheat dough in the usual manner and allow it to rise but once. To this dough is then added a soluble form of casein known as "Plasmon" (which contains 75 to 80 per cent of casein, 5 to 7 per cent of sodium carbonate, and 5 to 10 per cent of fat and milk sugar), a rye dough that has not been "set," and a dough made from wheat groats. Bread so made, it is claimed, has all the virtues of pure wheat or rye bread without their disadvantages. Another casein-containing bread is described[36] as being made of a gluten-rich flour, yeast and water. Following a preliminary fermentation, more of the same flour is added together with salt and casein. The dough is allowed to rise again and then baked.

A flour mixture[19] for use in the preparation of muffins or bread of low carbohydrate content or food value, but supplying bulk, is de-

scribed as consisting of casein, carbohydrate-free bran and soybean, a leavening agent and flavoring. It is called Curdolac-Casein-Bran Improved Flour. Its composition is protein 38 per cent, fat 9 per cent, carbohydrate 4.5 per cent, fiber 6.5 per cent, ash 7.8 per cent, and moisture 4.9 per cent. For the preparation of a carbohydrate-free bread, muffins, cake, etc., and for use in diets in general in which a relatively low carbohydrate content is desired, a somewhat similar mixture,[19] Curdolac Casein Compound, is available. Its approximate composition follows: protein 42.7 per cent, fat 0.75 per cent, nitrogen-free extract 42.35 per cent, ash 5.6 per cent, fiber 3.36 per cent, and moisture 5.23 per cent.

Schrederseker's[64] baking preparation is made by curdling skim milk with rennet and incorporating liquid egg yolk with the coagulum.

Egg Substitutes

Mixtures appeared on the market some years ago purporting to replace eggs in baking and many exaggerated statements were made regarding the value of these products as economical substitutes for whole eggs. The price of eggs at that time made housewives particularly susceptible to the implied suggestion that a few ounces of this yellow powder represented the nutritive value of two or three dozen eggs. According to La Wall[44] no other food substitutes sprang into prominence with such rapidity and with so little merit or legitimate warrant for their manufacture and sale.

Chemical analyses[43] indicated that some form of starch, usually cornstarch and occasionally rice starch, formed the major constituent of nearly every one of these egg substitutes. In addition to the starchy ingredients, one or more of the following were found: casein, gelatin, milk powder, sodium phosphate, sodium chloride, sodium bicarbonate, and in a few cases a little powdered egg albumin and rarely desiccated egg. Baking tests[3] made with these so-called egg substitutes showed the superiority of the egg for culinary purposes and the falsity of such positive assertions as "gives better results than eggs," "one scant teaspoon represents one fresh egg," etc.

Legislative action by many states concerning the sale of such preparations proved a deterrent to their acceptance by the housekeeper. Moreover, the federal requirement[77] that the words "egg powder" must not appear upon a product that contains no eggs, and that a product which is labeled to indicate that it will take the place of eggs must possess the properties of eggs, both in respect to food value and baking qualities, eventually relegated to history a food product, the suppression of whose sale is another milestone in the pure food movement.

A skim-milk product intended for use by bakers as an egg substitute is described by Bernstein.[10] Skim milk is heated to 95°C., after which

flour is added and the casein, containing also albumin, is incorporated with it by precipitation. This procedure seems to have been anticipated by another food product,[9] made from skim-milk casein coagulated in the presence of flour. Upon removal of the whey it is claimed that a light, readily digestible product is obtained which has no great influence on the flavor of breads to which it has been added.

A somewhat similar preparation is described by Dunham.[22] It consists of casein, an alkali, desiccated milk, egg albumin and sodium chloride and is claimed to be "perfectly soluble in hot or cold water or milk" and almost entirely free from odor.

Shortenings and Oleomargarine Manufacture

The problem of so incorporating the ingredients of an oleomargarine that it will respond on heating to the well-known foam test for butter is claimed to have been solved.[63] A non-gelatinizing solution of casein, made by heating casein with an alkali bicarbonate and then treating it with an equal amount of sodium chloride, is said to be capable of so emulsifying and binding the fats and water of the oleomargarine that its usual sputtering and grease-like action in a heated pan disappears. It is further stated that the function of the alkali bicarbonate is to cause the foaming and that the appearance of the brown color is due to sugar added as such or through the medium of the casein used. Although these claims can probably be accepted with some reservations, the use of casein in this direction apparently has its American counterpart in the practice of adding milk to oleomargarine not only for whatever nutritive value may be gained thereby, but also because of the emulsifying properties of casein and other constituents of milk.

Monhaupt's[51] milk-like solution, prepared by mixing finely comminuted casein with magnesium oxide and water, is said to be suitable for use in foods and confections. A strong solution so prepared may be used, it is claimed, as a substitute for white of egg or, with the addition of fat, as a "cream"; and in that form it is further said to be suitable as the major ingredient in the manufacture of an artificial milk and an oleomargarine.

Of historical interest only is the procedure of Adams[1] for preparing a solid oleaginous compound from a fatty oil and an alkaline solution of casein in the proportion of 60 parts of the former to 7 parts of the latter. The product is claimed to have the appearance of lard.

A process for the production of a food-fat mixture, which consists in emulsifying a fat or an oil with milk and adding to the resulting mixture a solution containing casein, sodium or potassium carbonate and a carbohydrate, such as glucose, is described by Schmidt.[62] The resulting products are claimed to have the properties of butter in that

they may be spread; they foam and become brown, on being heated, without splashing.

A composition comprising an intimate mixture of casein and a fatty oil which is suitable for use in cooking and other purposes in the presence of flour and baking powder is described by Dunham.[25] To prepare it, ordinary commercial casein is dissolved in water in the presence of an alkali. Cocoanut, or other "ingestible" oil is then stirred into it. The resulting emulsion is homogenized, after which sufficient acid is added to curdle the mixture. The precipitated material is removed, dried, and packaged.

A process of providing a food product is described by Epstein.[29] To wet, pressed casein (80 parts) there is added a fatty oil mixture having a melting point lying between 76° and 98°F.—cocoanut oil (18.7 parts) and hydrogenated cottonseed oil (1.3 parts) is cited as example —but sufficiently hard to be co-mingled and comminuted with the casein and to be molded into a relatively firm and homogeneous mass. A similar product, made from purified casein (rather than cottage cheese, because of claimed superior palatability and nutritional value) and cocoanut oil, is described by Sokolsky.[67]

Ice-Cream Mix Ingredients

Skim-milk powder emulsified with butter fat is widely used in ice cream. Letters patent have been granted Turney[75] on the use of casein as an ice-cream filler. To that end the swelling of the casein in the milk used is induced by pepsin, rennet, or acid. The swollen particles of casein are then "semidissolved," it is stated, by the addition of lime and sodium bicarbonate. Sugar is also added.

The body and texture of ice cream are improved by replacing dry skim milk with sodium caseinate sols. Studies made by Bird and associates[12] show that, depending on the composition of the mix, a 2.5 to 5 per cent replacement is advantageous. From the standpoint of flavor improvement, a progressive improvement by replacement of the former with the latter to the extent of 3 to 4 per cent was observed. Entering the picture also as a contributing factor to those ends is careful pH control as requisite in the preparation of the sol in question.

The use of soluble casein as a means of increasing the serum solids content without a concomitant increase in lactose content to a point where sandiness would occur appears to be the basis of a process for making an improved ice cream patented by Zoller.[80] This food product contains as principal constituents butter fat, milk solids-not-fat, unhydrolyzed alkali caseinate and sweetening material. The ice cream produced from this mix will be, it is claimed, of moderate butter-fat content and will have ample body and food value, marked smoothness to

the taste, and is capable of retaining this latter characteristic without the development of sandiness during prolonged cold storage. In this connection it may be timely to call attention to the fact that the preparation of a high-quality casein for use in ice-cream mixes, whose whipping properties may be controlled at will when the former has been incorporated, is described by Dahlberg.[20]

Soup Tablets

Soup tablets (Suppenwürzen), bouillon cubes, and similar forms of concentrated foods for preparing soups with a minimum of effort consist only in part of meat extractives. The ingredients of course are varied to suit the particular formula of the manufacturer. In Germany it is the practice to use casein as the major constituent of the fluid and semisolid products of this type.

Miscellaneous Uses

A casein phosphate preparation made from precipitated casein and phosphoric acid or monocalcium phosphate finds use, on desiccation, as a constituent of baking powder.[35] Somewhat similar products have been described by Just;[40] but Teller,[71] avoiding "ingredients that are foreign to the human body" claims a leavening agent containing a mixture of casein and hydrochloric acid "in lax chemical combination and in substantially dry and pulverulent form with an intimate mechanical mixture of sodium bicarbonate." Of more recent date than the foregoing, is Thatcher's composition,[72] a mixture of approximately 67 parts cream of tartar, 30 parts of sodium bicarbonate, 4 parts of milk sugar and 4 to 10 parts of finely powdered casein.

The clarifying properties of casein have been studied by Martin and Castaing[49] who observed that it can be used effectively for the preventive treatment, and to some extent as a remedial measure, in wines that are sensitive to maderization. The actual volume subjected to this treatment should be relatively small because of the difficulty of rapidly injecting the clarifying agent and distributing it thoroughly throughout the whole volume of the wine. A temperature of 10° to 15°C. is recommended and a 24-hour settling period should elapse before filtering. The removal of iron to a sufficient extent to reduce considerably the danger of "casse" can also be effected, it is stated, provided the wine is first well aerated and the casein is very rapidly distributed throughout the whole.

What appears to have been an early attempt at utilizing whey is seen in a process described in a patent issued to Rehnström[58] for preparing a food from milk curd. After coagulation of the milk and the removal of the casein, a "greater or smaller" part of the whey is evaporated to a desired volume. The casein is then intimately mixed with

this concentrate, after which the mass is evaporated to dryness and comminuted.

A method of making an edible alkali caseinate either from fresh curd casein prepared from skim milk by the so-called grain-curd process or from specially precipitated hydrochloric acid casein in dry form is described by Zoller.[81] In preparing this substance, a liquid suspension of the casein in the presence of a weak solution of alkali phosphate acting as a buffer is brought into solution with sodium hydroxide, or other suitable alkali, without, however, using an excess of the base. Describing his technic of controlling the formation of alkali caseinate as the "lacto-hydric method," the patentee claims that thereby alkali cleavage of the loosely bound sulfur, phosphorus, and nitrogen from the casein molecule is prevented, and that where such hydrolysis occurs toxic substances are produced with the result that the product in question is not suitable for use as a food. When properly made, this caseinate is said to be water-soluble and to possess a stability, without the use of preservatives, that is superior to other compounds of this kind. Soluble "casein sodium" is sometimes added to cocoa, and a readily soluble and assimilable, product is made from casein by the admixture of common salt, the mixture being heated to about 110°C, in order to induce, it is claimed, hydrolysis of the former.[53]

Medicinal Uses*

Marketed under various names,[19] as "Casec," "Larosan-Roche" and "Protolac" is calcium caseinate (*calcii caseinas*). It is the casein of cow's milk rendered partially soluble by combination with calcium and containing not less than 1.4 nor more than 2.1 per cent of calcium. It is a yellowish-white, fluffy powder, free from "sour" or rancid odor, has a characteristic slightly saline taste and is said to be permanent in the air. On being agitated with warm water, it forms a pseudo-solution or turbid suspension. It is used to increase the protein content of milk in order to obtain a product that is relatively low in fat and lactose but high in calcium salts. When added to gruels and other liquid foods to modify them, it is often used in this form in the treatment of the diarrheal diseases of infancy.

The sodium salt of casein bears the trade name of "Nutrose."[57] A similar American-made product bears the name "Nucleo." The former is a white, inodorous, tasteless powder containing about 65 per cent protein. It is slightly soluble in cold water, but very soluble in warm

* This material has been constructed in part from, and in some instances represents verbatim reproduction of, Chapter 8, *Casein in Medicine*, of the first edition (1927) of this work. Full credit, of course, rests with the original author, Peter Masucci. The writer claims none for himself. It seemed desirable to combine the original chapters in order to avoid duplication of material.

water. This product, in milk or water, is administered in large doses as a non-irritant nutrient in the treatment of convalescent, undernourished, or anemic patients.[4]

The manufacture of the latter is substantially as follows: The curd of fresh skim milk is precipitated with just sufficient hydrochloric acid to secure a clear separation. The curd is then washed repeatedly and redissolved with a minimum proportion of sodium bicarbonate. It is then precipitated again with hydrochloric acid, thoroughly washed, and again dissolved with a minimum proportion of sodium bicarbonate. The product is then diluted to about the consistency of milk in respect to solids content, and dried. Its protein content is approximately 81 per cent, its alkalinity, as Na_2O, 0.04 per cent.

The ammonium salt of casein is known as "Eucasein."[57] It finds use as a nutrient for consumptives and those suffering from stomach and lung trouble and is usually administered in hot coffee, chocolate or soup.[4] This product is made by passing ammonia over dry, finely powdered casein until tests show it to be almost completely soluble in water.

Another sodium preparation of this type bears the name "Plasmon."[4] It is made by mixing casein with sodium carbonate in an amount to render the former soluble. The mixture is twice heated to 90°C. in a suitable vessel, after which carbon dioxide is passed through the mass. The whole is then dried. It is sold as a light-yellow powder, possesses a slight, sweet, milk-like taste, and is soluble in hot water. When treated with insufficient water to effect solution, it swells and may even form a gel. Although still widely advertised in Germany as a food ("Plasmon-Milcheiweiss") which may be obtained admixed with cacao, zwieback, oatmeal, pastry, etc., it is probably not incorrect to include it here among the proprietary preparations. It appears to be a borderline case.

Compounds of casein with lithium have also been described. Although the type of medication for which they were intended may now be falling into disuse, yet it is perhaps not without interest to recall that such a preparation with lithium triphosphate[79] was once described. The patent in question claims "a tasteless remedy having nutritional properties for use in the treatment of gout or similar diseases which are commonly treated with lithium waters."

Casein can be made to combine also with the naturally occurring bases, the alkaloids.[73] The resulting water-soluble or alkali-soluble complexes, as the case may be, are deemed to be useful, it is said, in medicine since the casein residue seems to ameliorate some of the undesirable effects of the alkaloid without materially affecting its specific action. Morphine and caffeine preparations are made by triturating the alkaloid with casein in the presence of warm alcohol. The former product is water-soluble;

the latter alkali-soluble. An alkali-soluble quinine trihydrate compound with casein is prepared similarly in the presence of water.

Characteristic of the glycerophosphate complexes with casein is one sold under the proprietary name of "Sanatogen" as food and tonic. This product contains about 90 per cent casein and about 5 per cent of sodium glycerophosphate. It is made by triturating the casein with the glycerophosphate and then adding about 0.5 per cent of sodium bicarbonate and an equal quantity of sodium chloride. The resulting mixture is dried at a low temperature and comminuted to a fairly uniform size. In the same class and closely related to "Sanatogen" is "Casein phosphorol."[4] It finds a similar use. Relevant products and processes are also those of Bauer and Busch,[8] Dunham,[24] and Frey.[30] In an example cited by the latter, casein (60 parts) in the presence of water (800 parts) is dissolved in sodium pyrophosphate (112 parts) and calcium chloride solution. A ferric salt may be substituted for the latter.

Also advertised as a food and tonic is a preparation known as "Sanose."[4] Casein (80 per cent) and albumoses (20 per cent) are its major constituents. It is a white, odorless, tasteless powder which forms a milky suspension with water.

Of the several heavy metals that can be made to combine with casein, those with silver, mercury, iron, and bismuth hold a therapeutic interest. Silver compounds of casein are used in medicine as substitutes for silver nitrate which, although highly efficient as an antiseptic yet has a limited use because of undesirable side reactions, such as irritation, astringency and corrosion. It is known that the high antiseptic and irritant action of silver nitrate is, in the main, due to free silver ions. This principle is utilized in the preparation of protein-silver compounds whereby products are obtained that combine a fair degree of antiseptic action with a much smaller degree, or even absence, of irritant action. This is accomplished by changing a very large part of the active silver ions into inert forms of silver, such as colloidal metallic silver, silver oxide, or indefinite compounds of silver with protein split products. In other words, the antiseptic action of colloidal protein-silver preparations is due to the presence of low concentrations of silver ions, a concentration so low as to be practically non-irritating, but sufficient to show distinct antiseptic activity when tested against certain microörganisms, as for example gonococci.

Pilcher and Sollmann,[55] utilizing the fact that silver ions restrain the growth of yeast cells, have developed a method for measuring the free silver ions in protein-silver preparations and allied products. Application of their technic to the determination of the degree of restraint in aqueous solution of the latter divides them into four classes, *viz.*, "protargin" strong or mild, "collargol" and electric. The last two are not pertinent to this review, however, since they do not contain casein.

Protargin strong compounds contain the lowest percentage of silver, not less than 7.5 nor more than 8.5 per cent; but they have the strongest germicidal action. They are distinctly irritant and are intermediate in their therapeutic action between silver nitrate and protargin mild. Listed in "New and Non-official Remedies" (1925) in this group are "proganol," "protargentum" and "protargol." They are not casein-containing preparations.

The mild type protargin compounds contain from 19 to 25 per cent of silver. They are quite non-irritant because of the fact that they contain, when in solution, fewer silver ions. Of the various representatives of this type, only one known as "cargentos" is made from casein.

Cargentos is a casein-silver compound containing 20 to 25 per cent of metallic silver and 50 to 60 per cent of protein in the form of casein-split products. It is made by adding moist silver oxide to a partially hydrolyzed, low-ash casein, the hydrolysis being brought about by means of an alkali. The colloidal suspension is heated until no precipitate is obtained on the addition of a solution of sodium chloride to a test portion. It is then evaporated to dryness. This product is marketed in the form of black, non-hygroscopic scales having a metallic luster. It mixes readily with water with the formation of a clear, colloidal suspension, and contains no appreciable quantities of ionizable silver.

This preparation is used therapeutically on mucous membranes of the body, such as ear, eye, nose, throat, rectum, etc., for its antiseptic and germicidal value. In 5 to 50 per cent solution it has been used with satisfactory results in the treatment of inflammations.

A compound of casein and silver containing about 42 per cent of metallic silver is known as "Argonin."[4] It approaches in composition a true silver caseinate and, therefore, is soluble with difficulty in cold water. A fine, filtered 1 to 3 per cent suspension is used therapeutically in the treatment of gonorrhea and eye infections.

The situation that obtains in the case of therapeutically active silver salts in respect to corrosiveness, irritations, etc., finds its counterpart in the mercury salts. Several mercury-casein compounds are used in therapeutics. A caseinate is made by precipitating a solution of the neutral sodium salt and mercuric chloride with alcohol, or by evaporating the mixture. Another such preparation has been made by allowing a neutral solution of either the sodium or the potassium compound to react with mercuric iodide solution. The content of mercuric iodide of the final product is stated to lie between 15 and 20 per cent. None of this type of preparation, however, has found extensive use. Other mercury complexes have found greater favor.

Iron-casein compounds have been developed for the purpose of eliminating the astringent taste and local irritant action of simple iron solu-

tions. There is no evidence that they are compounds of definite chemical composition; they do not give the ordinary tests for ferrous or ferric ions; they are not decomposed by dilute acids or in the process of gastric digestion and are slowly soluble in dilute alkalies. They find some use in the treatment of anemia and chlorosis.

In this group falls a so-called ferric nucleo-albuminate, or ferric caseinate,[64] of 2.5 per cent iron content which is made by adding a freshly prepared solution of ferrous lactate to calcium caseinate. It is a water-insoluble, odorless, tasteless powder. Weak alkalies and ammonia dissolve it. This iron preparation is recommended as an easily absorbed tonic. Therapeutically similar to other masked organic iron preparations as to use is another proprietary compound of this type named "triferrin."[4] It is made by digesting casein with pepsin and precipitating the resulting soluble derivatives with a ferric salt. It is a reddish-brown powder which is insoluble in water but soluble in weak alkalies. Its iron content varies between 16 and 22 per cent. Phosphorus is present to the extent of 2.5 per cent; nitrogen 9 per cent.

Recommended wherever blood impoverishment exists, either as deficiency of hemoglobin or diminution of red blood corpuscles, is an iron nucleo-protein, "proferrin" N. N. R. It is a compound of iron and casein made by heating an alkaline solution of casein with an iron salt. The mixture is then treated with acetic acid until precipitation is complete. The finished product is an almost odorless and tasteless, water- and dilute acid-insoluble brown powder. Alkalies slowly dissolve it. It contains about 10 per cent of iron and a small proportion (0.5 per cent) of phosphorus. Proferrin is not affected by the gastric juice, does not precipitate albumin, and has no astringent action on the stomach. It is readily decomposed in the intestine and is rapidly absorbed.

A compound of casein with aluminum,[4] present to the extent of about 5 per cent, was once used medicinally as a remedy for intestinal catarrh because of the astringent properties of aluminum. It is insoluble in water but sparingly so in saliva. In dilute acids it is quite soluble. This preparation has, apparently, not withstood the competition of bismuth salts in this field of medication.

A number of compounds containing bismuth oxide have been prepared with albumin or casein as a base. In general, they are insoluble, possess a low antiseptic power and show a slight germicidal activity. Their action is said to be a mechanical one in that they protect inflamed or irritated surfaces. When dusted on wounds, they form a crust beneath which healing takes place. This is brought about by the very fine state of subdivision of the bismuth compound which possesses marked drying properties.

In connection with this brief discussion of the compounds of casein with the heavy metals, it may be timely to make reference to a patent

issued to Dunham[27] for the preparation of casein compounds allegedly suitable for coatings, plant foods, glues, and medicinal purposes. Homogeneous combinations of acid-precipitated casein with a neutral salt or hydroxide such as iron, copper, bismuth, nickel, zinc, etc., or with an ammonium salt, such as the acetate, not sufficiently alkaline to constitute a solubilizing agent for the casein, but capable of reacting with it, are extruded under heavy pressure to form a product which may be comminuted.

Forming the basis of a dusting powder used as an effective astringent, alterative, and healing dressing for wounds, ulcerations, and skin diseases is the bismuth-casein compound bismuth formic iodide. The iodine exerts a slight but effective antiseptic action and in so doing stimulates phagocytosis and diminishes the secretion of the wounds. The bismuth exerts a mechanical protective action, it is said, and thus contributes to the drying property of the powder. This preparation is made by adding a solution of potassium iodide to a solution of casein and treating the mixture with a bismuth salt. The resulting precipitate is washed and then allowed to react with formaldehyde. The final product is a water-insoluble, yellowish powder containing in organic combination about 1.4 per cent iodine.

The fact that casein in solution will form substitution or addition compounds with such widely different substances as halogens, tannins, and formaldehyde has been made use of in a technical way. With iodine casein forms compounds that are extensively used in medicine. Preparations of this sort appear to be less irritating to the digestive tract and do not show the sharp, disagreeable symptoms of iodine, as do the metallic iodides, nor are they germicidal because the iodine is not organically bound. A preparation in point is iodo-casein or casein iodine N. N. R., which is made by treating a solution of sodium caseinate with iodine and potassium iodide. Acetic acid is added until precipitation is complete, after which the latter is washed free of iodine, collected and thoroughly dried. It is a water-insoluble brownish-yellow powder of about 18 per cent iodine content, practically without odor and taste, and slightly soluble in dilute acid solutions. This preparation undergoes practically no change in the stomach but the alkaline intestinal secretions completely dissolve and digest it, it is said. Iodo-casein is recommended as a substitute for inorganic iodides in the prevention and treatment of simple or endemic goiter.

Solutions of caseinates react with formaldehyde to form insoluble compounds. The formaldehyde condenses with the amino groups in the casein molecule, thus forming methylene linkages, water being eliminated in the reaction. The effect of this condensation is an increase in the acidic character of the carboxyl group and a decrease in the basicity of the casein molecule. An example of this reaction is the proprietary

preparation "Proteol," which finds use as an antiseptic in the treatment of wounds. Although ordinarily an insoluble, tasteless yellow powder, it may also be prepared in the form of a thick paste, in which case it is used on cuts and bruises as an elastic coating to prevent bacterial infection. A preparation of somewhat similar nature is described by Durkoff.[28] In this instance, formaldehyde is added to a solution of casein in sodium hydroxide; this is followed by iodine in potassium iodide solution. The mixture is then precipitated with hydrochloric acid and the product collected, dried and powdered.

Casein, like gelatin and albumin, precipitates tannin from solution. Little is known, however, about the chemistry of the resulting combination. Since the compounds of this which have therapeutic interest are made by precipitating tannic acid with a solution of sodium caseinate, it is very probable that the reaction product is of a physical nature, perhaps a loose "adsorption" compound, *caseinum tannatum.* Inasmuch as both the colloidal suspension of casein and that of tannin bear negative charges, they cannot combine in the same manner as two oppositely charged ions would do.

The medicinal value of these preparations lies in the fact that tannic acid in uncombined form, when taken internally, dissolves rapidly in the stomach producing disagreeable reactions, such as irritation or nausea; but when in protein combination, because of the sparing solubility of the product in water and its almost complete in-olubility in dilute alkalies, such as sodium bicarbonate, it exhibits no such behavior. Therefore, when taken internally there is little action in the stomach but much greater action in the intestines. The casein-tannic preparations that are used medicinally are intended primarily as intestinal astringents in the treatment of diarrhoea. An example of this type is "tannocasein."[37] It is made by adding tannic acid and formaldehyde to a solution of sodium caseinate, treating the mixture with dilute hydrochloric acid, drying the mixture and comminuting it. Another example is "protan" N. N. R., a combination of tannic acid and an alkaline solution of casein. It contains about 50 per cent of tannin and about 45 per cent of casein. It is not more than 10 per cent soluble in water, not more than 25 per cent soluble in dilute (0.08*N*) hydrochloric acid, and about 80 per cent soluble in 1 per cent sodium bicarbonate solution. This preparation, it is said, has an astringent action throughout the entire intestinal tract.

Casein solutions, either acid or alkaline, hydrate well and form colloidal solutions which yield excellent emulsions. These emulsions are of the oil-in-water type. In this respect casein behaves similarly to gelatin, albumin, saponin, sodium oleate and other alkali soaps, but differently from gum dammar, lanolin, calcium oleate and heavy metal soaps, which yield emulsions of the water-in-oil type. In spite of the

fact that casein-containing emulsions are more stable and generally superior to those made with acacia and are deemed to be more readily retained by the stomach, yet, as an emulsifier, casein appears to be used only in a rather limited way in the manufacture of pharmaceutical preparations. The probable reason for this is that, when in solution, it is rather vulnerable to microbiological attack and, unless the preparation in question contains an antiseptic, it suffers decomposition in a short time.

The emulsifying and protective power of casein has been used in the development of antiseptic and mildly germicidal silver emulsions much superior to the ointments generally used. The reasons for this may be found in the differences that obtain between the emulsion types in question. Ointments are in reality emulsions of the water-in-oil type in which the external phase is their base which may be oil, petrolatum or lanolin. The dispersed phase is the water in which is dissolved the active germicidal ingredient. The oily base, and not the antiseptic, comes in contact with the surface of the wound and the bases of ointments are only indirectly or mechanically antiseptic in that they exclude air, dust and organisms from the area covered by them. Their antiseptic or germicidal value depends on the drug that is incorporated in them. On the other hand when casein is used as emulsifying agent the conditions are reversed, for the antiseptic is dissolved in the water, which then becomes the external phase while the inert base forms the dispersed phase. The antiseptic, therefore, comes in contact with the surface of the wound and hence becomes effective.

Casein-containing emulsions of this type are the preparations known as "Cargel" and "Arkase." Both are silver-containing preparations. A mixture of alkaline and alkali-earth caseinates is the emulsifier used in the former. Its base is lanolin which tends to produce an emulsion of the opposite type but, since casein is more strongly hydrated, the oil-in-water type prevails. The active antiseptic of this preparation is one per cent metallic silver added to it in the form of a colloidal solution which enters the external, or water phase by virtue of which it can readily act upon the microörganisms. Standard suspensions of bacteria, immersed in Cargel, are killed in 3 to 5 minutes, it is said. It inhibits the growth of yeast to the same degree as a 5 per cent solution of a Protargin Mild silver salt. Unlike ointments of the water-in-oil type, this emulsion mixes readily with water and can easily be washed off from the applied area. The emollient properties of the preparation make the action of the silver comparatively non-irritating so that it does not interfere with cellular activity or normal secretion. On application to a wound or other surface, it forms a smooth, dry coating because of the casein that it contains. It is useful in the treatment of wounds, abrasions

and burns, it is claimed, where infection must be checked and the surface protected.

Resembling "Cargel" in its action as a local antiseptic is "Arkase." It has been used quite extensively in skin eruptions and also in the treatment of nose and throat infections. Its germicidal value is equal to that of a 1-to-75 phenol solution. This preparation is claimed to possess superior penetrating powers in respect to the skin and mucous membranes.

Casein ointment (*unguentum caseini*,[37] *unguentum caseini Unna*[21]) is a mixture of alkali caseinate, glycerol, petrolatum and water to which phenol is added as antiseptic. *Unguentum caseini* (Beiersdorf)[4] is said to dry quickly when rubbed into the skin and to be easily removed with water. It may be incorporated with a large variety of medicinal substances, such as zinc oxide, resorcinol, pyrogallol and ichthyol, but is incompatible with acids and salts. It is best prepared, according to the United States Dispensatory[21] by dissolving 34.5 parts of potassium hydroxide and 8.5 parts of sodium hydroxide in 5000 parts of water and then dissolving in this 1400 parts of casein. In this solution there are then dissolved 700 parts of glycerol and 50 parts of phenol, after which 2100 parts of white petrolatum and 50 parts of zinc oxide are incorporated with the whole which is finally made up with water to 10,000 parts.

Saccharated casein has sometimes been used to make emulsions with fatty oils. It is a white powder "prepared[18] by heating one gallon of cow's milk to 140°F., adding two fluid ounces of water of ammonia, allowing the whole to stand a day and separating the lower milky liquid from the oily liquid on top. The milky liquid (lacto-serum) is treated with acetic acid until the casein is precipitated. After washing the precipitate thoroughly with water at 104°F. it is collected on a muslin strainer and pressed; a weighed portion of the casein is dried and the percentage of moisture determined; the damp cake is then triturated with 3.5 oz. of powdered sugar and 8 parts of sodium bicarbonate for every 100 parts of (dry) casein. Prolonged trituration and the addition of more powdered sugar, until it amounts to 9 parts in 100, result in the formation of a paste, which must now be dried by a gentle heat not above 86° to 90°F. After complete drying it is powdered and sifted. To make a casein emulsion of a fixed oil 15 parts of the oil are gradually incorporated with a mucilage previously made with 15 parts of saccharated casein and 5 parts of water. When a perfect emulsion is formed, the other ingredients are added."

Freshly precipitated casein from milk forms the basis of certain non-greasy cosmetics known as "rolling creams" because when rubbed over the surface of the skin they take the dirt and dust out of the pores by rolling up in small particles which can be brushed off. They contain

zinc oxide and gum tragacanth or other suitable medicaments. For a typical formula the reader is referred to Remington's *Practice of Pharmacy* (eighth edition, page 2013).

Of historical interest is a "topical remedy" described by Troplowitz[74] which "yields unguentous emulsions with every kind of fat, which have the property of forming a drying, flexible and cooling cover upon the skin in a few minutes." It is a glycerine-containing ammonium caseinate containing borax, if desired.

Determination of Casein

The determination of casein in proprietary preparations rests upon its isolation from the substance in question and its calculation, as a pure protein, from the nitrogen content determined by the usual Kjeldahl technic ($N \times 6.38$). To that end a one-gram sample is dissolved in dilute potassium hydroxide solution; the casein is precipitated with dilute acetic acid; and the nitrogen content of the precipitate determined. The presence of other proteins besides casein requires a modification in procedure. In this case, the total nitrogen content must be determined, from which the casein nitrogen is subtracted in order to obtain the figure representing that due to other protein forms.

References

1. Adams, C., U. S. Pat. 709,291 (Sept. 16, 1902)
2. Anonymous, *Milch Ztg.*, **29**, 821 (1900)
3. Anonymous, *New Hampshire Board of Health, Quar. Bull.*, **5**, 57 (1917); *Kansas State Board of Health, Bull.*, **14**, 36 (1918)
4. Arends, G., and Keller, O., "Neue Arzneimittel und Spezialitäten," 7th ed., Berlin, Julius Springer, 1926
5. Backhaus, German Pat. 115,958 (Nov. 3, 1897)
6. Backhaus and Braun, R., *Ber. Landw. Inst. Univ. Königsberg*, **5**, 34 (1901); *J. Chem. Soc.* (abst.), **80 ii**, 529
7. Bailey, E. M., *Conn. Agr. Expt. Sta. Bull.*, **220**, 265 (1920)
8. Bauer, F. and Busch, A., U. S. Pat. 601,995 (Apr. 5, 1898)
9. Bernstein, A., German Pat. 98,322 (Aug. 10, 1897)
10. Bernstein, A., German Pat. 103,156 (Jan. 5, 1898); U. S. Pat. 603,165 (Apr. 26, 1898)
11. Beythien, A. and Hempel, H., *Pharm. Zentralb.*, **53**, 361 (1912)
12. Bird, E. W., Sadler, H. W., and Iverson, C. A., *Iowa Agr. Expt. Sta., Research Bull.*, **187**, 179 (1935)
13. Bosworth, A. W. and Chrysler, L. H., U. S. Pat. 1,563,891 (Dec. 1, 1925)
14. Bosworth, A. W., U. S. Pat. 1,341,040 (May 25, 1920)
15. Brandenburg, K., *Deut. Arch. klin. Med.*, **58**, 71 (1896)
16. Chem. Fabrik auf Aktien vorm. Schering, German Pat. 96,543 (Apr. 24, 1897)
17. Clark, W. M., Zoller, H. F., Dahlberg, A. O., and Weimar, A. C., *J. Ind. Eng. Chem.*, **12**, 1163 (1920)
18. Cook, E. F. and LaWall, C. H., Remington's "Practice of Pharmacy," 7th ed., 1660. Philadelphia, J. B. Lippincott, 1926
19. *Ibid.*, 8th ed., 1819, 1936
20. Dahlberg, A. C., U. S. Pat. 1,962,552 (June 12, 1934)

21. Dispensatory of the United States, 21st ed., 1405, Philadelphia, J. B. Lippincott, 1926
22. Dunham, A. A., U. S. Pat. 732,929 (July 7, 1903)
23. Dunham, A. A., U. S. Pat. 750,832 (Feb. 2, 1904)
24. Dunham, A. A., U. S. Pat. 1,080,204 (Dec. 2, 1913)
25. Dunham, H. V., British Pat. 169,276 (June 22, 1920); U. S. Pat. 1,431,937 (Oct. 17, 1922)
26. Dunham, H. V., British Pat. 180,018 (Feb. 12, 1921)
27. Dunham, H. V., U. S. Pat. 2,103,153 (Dec. 21, 1937)
28. Durkoff, H. C., U. S. Pat. 601,072 (Mar. 22, 1898)
29. Epstein, A. K., U. S. Pat. 1,676,138 (July 3, 1928)
30. Frey, A., U. S. Pat. 1,381,295 (June 14, 1921)
31. Funk, C. and Friedman, L., U. S. Pat. 1,505,551 (Aug. 19, 1924)
32. Funk, C., Paton, J. B., and Friedman, L., *J. Metabolic Research,* **3**, 1 (1923)
33. Hahn and Planitzer, Austria Pat. 3,886 (Dec. 15, 1900)
34. Hall, W. A., U. S. Pat. 746,502 (Dec. 8, 1903)
35. Hatmaker, British Pat. 19,691, 19,692 (Aug. 7, 1902)
36. Heudebert, German Pat. 156,797 (Jan. 6, 1903)
37. Hofman, J. J., *Pharm. Weekblad,* **73**, 420 (1936)
38. Hunziker, O. F., "Condensed Milk and Milk Powder," 4th ed., 547, LaGrange, Ill., 1926
39. Jolles, A., *Z. landw. Versuchsw.,* **7**, 515 (1904)
40. Just, J. A., U. S. Pat. 692,451, 692,452, 692,453 (Feb. 4, 1902)
41. König, J., *Milch Ztg.,* **31**, 759 (1902)
42. Kreis, H. and Iselin, E., *Mitt. Lebensmittelunters. Hygiene,* **21**, 234 (1930)
43. LaWall, C. H., *Am. Food J.,* **12**, 300 (1917)
44. LaWall, C. H., *Am. Food J.,* **13**, 664, 684 (1918)
45. Liebrecht, A., German Pat. 94,406 (May 1, 1896)
46. Mansfield, M., *Jahresbericht der Untersuchungsanstalt des allgem. österr. Apotheker-Vereins,* 7 (1907-08); *Z. Nahr. Genussm.,* **17**, 278 (1909)
47. Marcuse, G., *Arch. ges. Physiol. (Pflüger's),* **64**, 223 (1896)
48. Marcuse, G., *Arch. ges. Physiol. (Pflüger's),* **67**, 373 (1897)
49. Martin, R. and Castaing, *Ann. fals.,* **27**, 340 (1934)
50. Mitchell, H. S., *Am. J. Physiol.,* **74**, 359 (1925)
51. Monhaupt, M., U. S. Pat. 1,326,210 (Dec. 30, 1920)
52. Neukirch, H., *J. praticiens,* **39**, 602 (1925); *Chem. Abst.,* **20**, 934
53. Nyegaard and Co., Norway Pat. 24,246 (Nov. 3, 1910)
54. Osborne, T. B. and Mendel, L. B., *Z. physiol. Chem.,* **80**, 307 (1912)
55. Pilcher, J. D. and Sollmann, T., *J. Lab. Clin. Med.,* **8**, 301 (1923)
56. Racine, R., *Z. öffentl. Chem.,* **12**, 169 (1906); *Z. angew. Chem.,* **20**, 275 (1906)
57. Rakuzin, M. A. and Braudo, K., *Z. Untersuch. Lebensm.,* **52**, 396 (1926)
58. Rehnström, A. W., U. S. Pat. 446,887 (Feb. 24, 1891)
59. Revis, C. and Bolton, E. R., Allen's "Commercial Organic Analysis," 4th ed., **8**, p. 193, Philadelphia, Blakiston, 1913
60. Riegel, German Pat. 117,979 (May 31, 1900)
61. Scherer, R., "Lebensmittel deren Ersatzstoffe und künstliche Nährpräparate," p. 37, Vienna, Hartleben, 1919
62. Schmidt, P. A., U. S. Pat. 1,034,036 (July 30, 1912)
63. Schmitt, Austria Pat. 72,011 (Dec. 1, 1915)
64. Schrederseker, German Pat. 166,849 (Jan. 20, 1905)
65. Seaber, W. M., *Sanitary Record,* **49**, 20 (1912)
66. Sell, German Pat. 116,387 (Aug. 2, 1899)
67. Sokolsky, L., U. S. Pat. 1,676,166 (July 3, 1928)
68. Sorenson, J., *Creamery Milk Plant Monthly,* **8**, 33 (Mar. 1919)

69. Stevenson, A. F., Peck, G. C., and Rhynus, C. P., *U. S. Public Health Service, Public Health Reports, Reprint* 608, 2011 (1920)
70. Street, J. P., *J. Am. Med. Assoc.*, **63**, 1831 (1914)
71. Teller, G. L., U. S. Pat. 749,643 (Jan. 12, 1904)
72. Thatcher, H. D., U. S. Pat. 1,286,145 (Nov. 26, 1918)
73. Thomas, F., U. S. Pat. 626,110 (May 30, 1899)
74. Troplowitz, O., U. S. Pat. 527,906 (Oct. 23, 1894)
75. Turney, P. W., U. S. Pat. 1,424,602 (Aug. 1, 1922)
76. U. S. Dept. Agric., Office of Sec., Food Insp. Decision 199 (1926)
77. U. S. Dept. Agric., Bur. Chem., Service Reg. Annts., 3, item 23; 17, item 172; 24, item 329
78. Van Slyke, L. L. and Baker, J. C., *J. Biol. Chem.*, **35**, 127 (1918)
79. Wendt, G., U. S. Pat. 617,210 (Jan. 3, 1899)
80. Zoller, H. F., U. S. Pat. 1,598,033 (Aug. 31, 1926)
81. Zoller, H. F., U. S. Pat. 1,598,334 (Aug. 31, 1926)

Chapter 13

Miscellaneous Uses of Casein and Statistics

Besides the uses described in Chapters 7 to 12, casein finds application in many other industries. In hearings before the Joint Committee on Tariffs of the Congress in 1922 it was stated[49] that over 50 industries were using casein in material quantities. It is certain, however, that minute quantities of casein were considered "material" for the purposes of this statement. No doubt casein has been used at some time and to some extent in more than 50 industries but many of the uses were temporary or experimental and ultimately proved impracticable or uneconomical. On the other hand there has long been a tendency on the part of those interested in the production and sale of casein to regard too lightly those applications in which its consumption is necessarily small. In the aggregate such minor uses may be more important than is generally realized.

It has proved impossible to gauge the extent to which casein enters into most of the miscellaneous uses mentioned in this chapter because of contradictory reports received from those believed to be in a position to know about them. It therefore seems best to list all uses that have been seriously suggested, as far as they are known, even though some of them never have proved more than suggestions.

Insecticide Sprays

E. Sutermeister

Casein has found quite a wide application as a spreader for spray materials to be applied to fruit trees, vegetables, etc. It is a common observation that poisons applied as a spray without a spreader tend to roll off the surface of many kinds of foliage and fruit, and that the spray runs off the upper surfaces of twigs and branches, leaving them insufficiently covered. The thoroughness of application of such a spray is judged largely by the number and proximity of the spots of the dried spray material, and it is perfectly obvious that there are many interspaces that are apparently free from poison. This condition tends to limit the effectiveness of sprays and much study has been given to improving their physical properties so that better results can be obtained.

Robinson[45] states that an ideal spreader should cause a continuous film of poison to be deposited on the surface; it should increase adherence; it should not react chemically with the poison to form by-products that would cause foliage injury;[1] it should not diminish the toxic properties of the poison; and it should not cost too much. While it may be impossible to find a spreader that fulfills all these requirements it has been found that many substances effect great improvements over sprays made without spreaders. Spreaders may be divided into three classes; namely, soaps, synthetic wetting agents, and materials containing water-soluble or colloidal solutions of proteins. In general the soluble proteins are effective at much lower concentrations than the soaps.

Of the proteins casein has been much the most generally used. The first work of any importance with casein was done by the late Professor A. L. Lovett[34] of the Oregon Agricultural College, who in 1919 made orchard observations of the spreading qualities of various materials. By 1925 the use of casein had increased to an estimated consumption of 300,000 pounds annually. Although synthetic wetting agents have become widely used in recent years, a consumption of 1,000,000 pounds of casein annually is considered a reasonable guess at the present time by the Economic Entomologist of a middle western agricultural college.

There are several commercial brands of casein spreader on the market, most of which consist of finely ground casein mixed with about three times its weight of dry hydrated lime. This is convenient to use, but if desired an equally satisfactory mixture may be made by the consumer, the only requirements being that the hydrated lime shall be dry and finely divided, and that the casein shall be of good quality and shall be ground fine enough to pass at least a 100-mesh sieve. Such a mixture of the dry materials may be dissolved in a number of ways, all of which are designed to avoid the formation of lumps. It may be washed through a strainer into the sprayer tank while filling the latter with water; or it may be beaten into a small amount of water, much as flour is mixed, and subsequently diluted; or it may be shaken in a closed receptacle in the proportions of one pound of spreader to a gallon of water. Equally satisfactory results may be obtained by mixing 4 ounces of hydrated lime to a smooth paste with about an equal amount of water, diluting to one pint and stirring in 4 ounces of casein. Stirring must be vigorous and continued for at least five minutes, or until a thick, fluffy paste has been obtained; when diluted with an equal volume of water this is ready to be added to the other spray materials.

Spreaders prepared with lime possess the desirable property of drying to a practically insoluble film, which means that the poison is not easily washed off the fruit or foliage by rains. Where this property is not of such great importance a spreader may be made by dissolving the casein by means of sodium hydroxide, sodium carbonate, or sodium

bicarbonate. Dissolve the sodium hydroxide or other alkali in about a quart of water and sift a pound of the casein slowly into the solution, stirring meanwhile. Continue stirring until the casein has entirely dissolved, and add water to any desired consistency. This solution may be used as a spreader in the same concentration as the lime-casein; namely, about three-quarters of a pound to 100 gallons of spray. For this spreader a less soluble and coarser casein may be used, since the solvent power of the various compounds of soda is greater than that of hydrated lime.

The amount of lime-casein spreader to use is variously stated as three-quarters of a pound to two pounds of the dry mixture for 100 gallons of spray. The smaller quantities are apparently used with lead or calcium arsenate while the larger amounts are recommended for oil sprays. It is also used with Bordeaux mixture, nicotine sulfate, lime-sulfur, and other materials. To secure the best results many factors, such as the type of gun or nozzle, the kind of surface to be covered, the atmospheric or climatic conditions, etc., must be taken into account and the amount of spreader governed accordingly. Most of these points need to be settled by careful field observations before the general principles can be established with certainty.

A satisfactory oil-emulsion spray may be prepared by dissolving 1 pound of spreader in water, making up to 4 gallons in the sprayer tank and adding 8 gallons of oil. The pump is then started and the mixture sprayed back into the tank at a pressure between 200 and 300 pounds until a good emulsion is obtained. A stock oil-emulsion may be prepared from 50 gallons of oil, 25 gallons of water, 4 pounds of spreader and half a pound of copper sulfate. Dissolve the spreader in a little water and add the rest of the water, add the copper sulfate dissolved in a little water, and finally add the oil. Pump back into itself at high pressure until a good emulsion is obtained. In all such mixtures the spreader should never be added to the oil, but should first be dissolved in water. Commercial oil-emulsion sprays are on the market, most of which are licensed under a patent to Volck.[57]

The advantages claimed for lime-casein spreader are very numerous, among them being that it coats all parts of the twigs and foliage thoroughly; dries quickly; will not wash off with rain; reduces sunburn; avoids spotted fruit; permits fruit to color evenly; overcomes spray burning; keeps spray nozzles from clogging; keeps materials from settling in the spray tank and makes the spray more conspicuous so that covering can be observed better. The advantages and limitations of casein spreaders have been studied by various agricultural experiment stations.[1, 50]

After it was observed that solutions of proteins spread well on most surfaces the study of this class of substance was extended to include

albumin, pepsin, hot-water extracts of dried blood, sage, clover, vetch, alfalfa, etc. These were found to spread well on all surfaces tested, thus adding to the evidence that soluble protein is the active agent. Another possible substitute for lime-casein spreader is skim milk or some of its products, such as dried or condensed skim milk. Tests in the laboratory and a few field experiments indicate that 2 or 3 quarts of skim milk in 100 gallons of spray will give good results on many surfaces including the foliage of apple, pear, prune, peach, cherry, and cabbage, and apple and pear fruits. Equally good results were obtained with the dried product in equivalent amounts. With all skim-milk products it is necessary to add a few ounces of hydrated lime to each quart of milk, or its equivalent; if that is not done spreading will not result. These milk products are easily brought into solution and in this respect are preferable to commercial lime-casein, which requires much more careful handling in order to give satisfactory results in the sprayer.

Probably the most important point to consider in regard to spreaders is the cost. If this is low spreaders can be used to good advantage in most sprays, but if it is high they should be used only in those cases where the benefit derived is sufficiently great to justify the expense.

Textiles

L. A. Olney

Casein is not extensively used in the textile industry, although some of its properties render it adaptable for various processes of textile printing and finishing. The value of its application lies in the fact that casein may be precipitated from solutions of certain caseinates, particularly those of sodium and ammonium, and rendered still further insoluble by the action of formaldehyde.

It is in the pigment style of calico printing, in the preparation of double cloths as an adhesive, and in the finishing and waterproofing of cotton material that casein has found its most extensive uses. It has also been suggested at different times that cotton material treated with casein might become sufficiently "animalized" to develop an affinity for acid dyes, similar to that of the animal fibers. Such a process does not appear to have been worked out at any time with sufficient success to have warranted its adoption by the trade.

An alkaline solution of casein has also been suggested as an aid to detergent action, but for this purpose it apparently has no advantages over the ordinary soaps, either as to cost or effectiveness.

Ever since the earliest days of calico printing, the so-called pigment style has been used. Through the adoption of this style, it is possible to print upon textile material various pigments and color lakes in con-

junction with some adhesive substance, which is of a more or less soluble nature while in the printing paste, and yet capable of being converted into a decidedly insoluble substance later. Albumin has always furnished a satisfactory substance for this purpose and being soluble in cold water it could readily be incorporated in sufficient quantity into the printing paste, together with a colored mineral pigment. After printing this color paste upon the cloth, a passage through a steam ager coagulates the albumin, and a colored print results which possesses fastness to washing and light, which are satisfactory for all ordinary requirements.

It is as a substitute for albumin that casein has been chiefly used in textile printing. The pigment to be used is incorporated with the casein, which has been dissolved in a warm, slightly alkaline solution, and with the addition of proper thickeners and gums this may be made into a paste suitable for textile printing. The printed material is then dried and passed through a solution of formaldehyde or through a steam ager containing steam and formaldehyde vapors. In this way the casein is rendered sufficiently insoluble to hold the pigment upon the fiber. When a fabric printed with a pigment and casein paste is exposed to formaldehyde vapor alone there is danger that only a surface combination will be made, whereas if the formaldehyde is introduced directly into the paste a more permanent fixation will result. It requires considerable experimentation and skill, however, to do this in such a way that the coagulating effect will not take place until the goods are steamed or exposed to the air for some time. The addition of hexamethylenetetramine to the casein printing paste is said to be beneficial. Prints produced in this way are not so fast to an alkaline soap washing and not so resistant to various other reagents as albumin prints. This, together with the fact that the use of casein involves more work and complications, has prevented it from making any great headway in replacing the albumin method.

In the allied industry of wall-paper printing, which is quite closely related in certain respects to textile printing, mineral pigments and color lakes have been successfully printed using casein with the addition of a small percentage of formaldehyde to the color paste just before printing.

As a finishing material casein has a tendency to make the goods rather too stiff, but for the finishing of light-weight materials, such as organdies, it has been used with some success.

A somewhat different use of casein in the textile printing industry has been in connection with color furnisher brushes. The brushes are immersed in a solution of casein and formaldehyde and the former becomes fixed upon the fibers, either through an air drying or steaming. The surface of the fibers of the brushes is thus protected, and becomes more durable.

In the manufacture of double cloths where a piece of rather firm

material like duck or heavy twill has to be backed by a softer fabric, as, for instance, cotton flannel, casein has been used to some advantage as an adhesive in holding the two fabrics together. For this purpose casein is commonly dissolved with a mild alkali, as, for instance, borax or ammonium hydroxide. The latter has the advantage of evaporating during the process of drying and thus aids somewhat in developing insolubility and adhesive character of the casein. A small amount of castor oil, or some other oil softener, added to the casein solution improves the pliability and softness of the finished fabric.

In the preparation of material waterproofed with casein, the cloth is first prepared with a solution of some aluminum salt, preferably the acetate, and thoroughly dried. The cloth thus prepared is then passed through a solution of casein in ammonium hydroxide and a little soap, and then again dried. A final passage through another solution of an aluminum salt, as, for instance, alum, is also an advantage. In this way a combination of aluminum soap and aluminum caseinate is formed which produces a shower-proof effect upon the material.

In the manufacture of emery cloth casein has been found to be satisfactory for backing the cloth before applying the glue.

Casein is also said to be used[39] for giving a high gloss to fabrics, giving them metallic finishes, making mercerized crepes, dressing linens, and for loading silk and cotton. Casein could be used as dope for tautening aircraft fabric for the wings and fuselage of airplanes, for which purpose it is more fire-resistant than cellulose acetate[29] but it does not seem to have been used commercially for that purpose.

The list of references at the end of the chapter includes some on casein in textiles.[4, 17, 27, 37, 42, 46, 53]

Soap and Cosmetics

When casein is used in soap it probably is primarily a filler.[4, 32] Though the claim is advanced that casein improves the detergent properties of soap,[16] others deny it and maintain instead that casein has a favorable action on the skin[2] or that it "neutralizes excess alkalinity."[32] It is also said to give an agreeable texture to the soap and to improve the bad odor of the fat. In neutral or slightly acid soaps, however, it tends to provoke rancidity. In preparing the soap the casein is dissolved by means of borax and soda and added to the hot soap before it goes to the frames. As much as 1 part of casein to 3 parts of soap may be used.[16] Soaps containing casein are sometimes sold as "milk soap" or "buttermilk soap." Casein probably adds nothing to the value of soap, though it may furnish much inspiration to the agent writing advertising copy for it. A review of patents on the use of casein in soap is given by Ohl.[41]

Casein is used to some extent in massage creams,[36] in perfume com-

positions, to which it is added for the purpose of retaining the perfume,[39] and in dermatologists' ointments.[6, v. II, p. 364]

Other Miscellaneous Uses

In photography casein has been proposed for use in sensitizing solutions,[6, v. II, p. 404] for making casein-pigment prints, in printing out papers, and for films. Its use apparently has never gone much beyond the experimental stage and about the only information available is that given in patent specifications.[9, 10, 19, 20, 22]

In the printing industries casein finds some uses in bookbinding and lithography and in the manufacture of aqueous inks such as intaglio inks.[27, 30, 51, 56] According to patent claims the vehicle of an intaglio ink may consist of an ammoniacal solution of casein and animal glue, an oxidizing agent such as potassium dichromate, linseed oil, a deodorant such as oil of citronella, and a hydrocarbon thinner.[30]

Although casein has no use in ordinary wax shoe polishes for black or tan shoes,[5] it is used in liquid cleaners and polishes for white shoes, where it serves as a binder for white pigments.

Casein may be used as a substitute for shellac for certain purposes, for example, as a fixative.[43] For this purpose it may be dissolved in water by means of alkaline salts, but certain organic bases are now being recommended instead. Among these are triethanolamine,[12] morpholine,[13] and Trigamine (a partly esterified polyglycol amine[23]). These organic bases make very mildly alkaline solutions of casein.

In certain processes of bleaching wood before applying "blonde" finishes, a solution of casein is used to make a viscous vehicle carrying the bleaching chemicals so that they can be brushed on like paint.[56a]

It is reported that small proportions of casein, up to 5 per cent, decrease the water required for normal consistency and hasten the time of set of portland cement.[25]

In the electroplating of cadmium, casein is sometimes used as an addition agent to improve the deposit.[8]

A tabulation of the uses of casein made by *Oil, Paint and Drug Reporter*[39] lists the following applications, most of which have not previously been mentioned in this book:

In chemical analysis as a reagent for formaldehyde, for tannin, and for the effectiveness of digestive ferments such as pepsin and trypsin.

In ceramics as an addition to increase the hardness of potteries and porcelains.

In chemical manufacture as the starting point for making acrolein compound and albumin.

In construction as an ingredient of compositions for damp-proofing walls, for insulating cements, mortars, and plaster-lath compositions.

In dye manufacture as an ingredient in color lakes, particularly in nonpoisonous rhodamine and eosine lakes.

In insulating coatings for electrical wiring and machinery.

In compositions used in making matches.

In fuels as a binder for briquettes of coal dust.

In making linoleum and oilcloth as an addition to linseed oil.

In anticorrosion preparations.

In antiradiation coverings for steam pipes.

In asbestos compositions for high-pressure steam gaskets.

In brake-shoe fillings and linings as a binder[33] and in brake linings.[28]

In cooperage for cask glazing and luting.

In compositions for making picture moldings.

In compositions containing colored micas.

In artists' materials as a priming for artists' canvas.

In compositions containing bituminous substances for road surfacing.[38]

In making liquid court plaster.

In making artificial horse hair.

In manufacturing ultramarine as a reagent for improving the dispersion of the pigment in linseed oil.[55]

In the form of formolactin (the compound with formaldehyde) as an antiseptic in paints for hospitals and dairies.

As an ingredient of paint removers.

As an ingredient in solidified compositions containing petroleum oils.

In celluloid as a substitute for camphor to make the product less inflammable.

In rubber as an ingredient of hard rubber and of latex compositions.[7]

In making wine, as a clarifying agent.

In foundry sands.

The list of references at the end of this chapter contains a number of general articles on the uses of casein in industry that are not cited in previous chapters.[3, 21, 24, 31, 35, 44, 48, 52, 54]

Statistics

The domestic production and imports of casein since 1916, as reported by the Bureau of Agricultural Economics of the United States Department of Agriculture and the Bureau of Foreign and Domestic Commerce of the Department of Commerce are given in Table 32, together with the total consumption and the consumption per capita. The total consumption is assumed to be the sum of the domestic production and imports, uncorrected for carry-over. For reasons that will appear in subsequent discussion, it is believed that domestic production and total consumption are somewhat larger than the figures given.

When due allowance is made for the business depression of 1930 to 1936 it appears that the per capita consumption of casein has more than doubled and perhaps nearly trebled since 1916. From a fifth of a pound per capita per year before 1919 consumption rose to a peak of roughly half a pound in 1930, fell again to a fifth of a pound in 1932, and then rose rapidly to 0.558 pound in 1937. The minimum in 1932 was nearly twice the minimum in the previous depression year 1921.

TABLE 32.—Domestic Production, Imports, and Consumption of Casein, 1916 to 1937

Year	Domestic production[a] (lbs.)	Imports[b] (lbs.)	Estimated consumption[c] (lbs.) Total	Per capita
1916	8,421,973	10,376,641	18,798,614	
1917	8,342,350	12,319,111	20,661,461	0.207
1918	8,675,504	12,106,855	20,782,359	
1919	14,407,394	8,125,175	22,532,569	0.216
1920	11,526,000	21,238,822	32,764,822	
1921	8,076,000	9,717,238	17,793,238	0.107
1922	6,927,000	14,342,498	21,269,498	
1923	14,548,000	26,489,992	41,037,992	0.370
1924	20,759,000	17,749,985	38,508,985	
1925	16,660,000	18,803,000	35,463,000	0.310
1926	16,953,000	26,281,000	42,234,000	
1927	18,033,000	24,209,000	42,242,000	0.360
1928	22,151,000	28,728,000	50,879,000	
1929	30,537,000	27,583,000	58,120,000	0.480
1930	41,965,000	18,499,000	60,464,000[d]	0.494
1931	35,335,000	3,503,000	38,838,000	0.314
1932	24,428,000	1,475,415	25,903,415	0.207
1933	24,087,000	8,319,810	32,406,810	0.257
1934	37,331,000	1,491,140	38,822,140	0.307
1935	37,638,000	3,230,000	40,868,000	0.320
1936	46,140,000	16,209,000	62,349,000	0.486
1937	67,467,000	5,210,000	72,677,000	0.558

[a] Domestic production from data of the Bureau of Agricultural Economics, United States Department of Agriculture.

[b] Imports from data of the Bureau of Foreign and Domestic Commerce, United States Department of Commerce. (Imports for 1916 to 1919 inclusive are for fiscal years, not for calendar years; imports for subsequent years and all data for domestic production are for calendar years.[15])

[c] Estimated consumption is the sum of domestic production and imports.

[d] Production and imports may have greatly exceeded consumption in 1930, the excess representing carry-over. One estimate of consumption in 1930 is 42,499,656 pounds,[39] which seems too low.

Before 1929 more casein was imported than was produced in the United States, except in 1919 and 1924. Since 1929 domestic production has always exceeded imports and in recent years has greatly exceeded them. The shrinkage in imports was hastened by an increase in duty from 2½ to 5½ cents a pound beginning in 1930, but improve-

ments in the quality and uniformity of domestic casein have likewise played an important part. Before the World War the principal supplier of imported casein was France; but since that time Argentina has been the chief source, supplying as much as 90 per cent of the imports. Other countries from which casein was imported between 1928 and 1934 were: Germany, Canada, Great Britain, New Zealand, Netherlands, and Denmark.[39]

The distribution of domestic production by states is indicated in Table 33. Although casein is consumed chiefly east of the Mississippi River, California was the largest producer during most of the time from 1920 to 1933. In 1920 New York was second, Pennsylvania and Illinois third and fourth, and Vermont and Wisconsin fifth and sixth. In 1923 Wisconsin began to forge ahead and between 1924 and 1929 Wisconsin, California, and New York vied with one another for the leadership. During the years of depression following 1929 the order remained California, Wisconsin, New York; but during the last few years Wisconsin seems to have definitely taken first place with California second and New York third. There has also been marked increase in production in Idaho, Illinois, Minnesota, Missouri, Washington, and Vermont.

In 1937 the Bureau of Agricultural Economics reported 624 factories manufacturing dried casein. In 1921 there were only 136 factories. The total production of 67.47 million pounds in 1937 varied seasonally as follows: January 4.24, February 4.18, March 6.62, April 7.42, May 8.82, June 10.08, July 6.99, August 5.04, September 4.29, October 3.74, November 2.85, December 3.20.

Statistics on the consumption of casein by industries are meager and perhaps unreliable. In the Biennial Census for 1931, under the heading of glue and gelatin, production of casein glue was reported as 3,200,747 pounds in 1927, 2,652,840 pounds in 1929, and 2,787,406 pounds in 1931. In the Biennial Census for 1935 the estimates for 1929 and 1931 were revised and reported as 6,760,000 and 4,787,000 pounds respectively and for 1935 production of 5,873,943 pounds was reported. Presumably the data refer to prepared casein glues, in which the casein probably would amount to roughly 60 per cent of the weight of the glue. In addition to the prepared glues, however, very considerable amounts of wet-mix casein glues were used during the period in question, for which the glue user bought casein that would not be reported as casein glue. An estimate of 5 or 6 million pounds of casein used in 1935 for making casein glue seems reasonable in the light of these data.

The Biennial Census for 1935 reported casein among the raw materials consumed in the manufacture of paper to the amount of 36,902,000 pounds in 1929 and 35,744,000 pounds in 1935. Paper coating therefore accounted for 63.5 per cent of the total consumption of casein in

TABLE 33.—Domestic Production of Casein by States, 1920 to 1937

in Millions of Pounds

Year	California	Idaho	Illinois	Michigan	Minnesota	New York	Pennsylvania	Vermont	Wisconsin	Other states	Total
1920	4.76	0.10	0.89	0.26	....	1.62	0.90	0.50	0.43	2.06	11.53
1921	2.72	0.03	0.13	....	0.23	1.01	0.42	0.83	0.36	2.35	8.08
1922	2.41	0.03	0.04	....	0.14	1.42	0.44	0.46	0.78	1.21	6.93
1923	4.75	0.10	0.15	0.14	0.63	2.49	0.62	0.94	3.06	1.69	14.55
1924	5.95	0.13	0.67	0.41	0.90	4.30	0.30	1.15	5.96	0.99	20.76
1925	4.78	0.22	0.52	0.33	1.04	4.36	0.26	1.29	3.09	0.76	16.66
1926	3.57	0.94	0.28	0.23	0.95	5.08	0.29	1.22	3.68	0.71	16.95
1927	4.28	0.59	0.36	0.26	0.77	4.86	0.21	1.44	4.65	0.60	18.03
1928	7.05	0.63	0.70	0.22	0.39	4.94	0.38	2.11	4.97	0.77	22.15
1929	7.61	1.02	1.45	0.42	1.24	3.87	0.06	2.54	9.16	3.15	30.54
1930	11.81	1.65	1.51	0.53	1.86	5.66	0.31	2.89	13.70	2.06	41.97
1931	11.32	1.34	1.82	0.20	0.35	5.16	1.30	2.72	10.04	1.09	35.33
1932	9.45	0.95	1.86	0.19	0.51	2.88	0.26	1.75	6.30	0.28	24.43
1933	9.14	1.11	1.98	0.31	0.65	3.57	0.18	2.75	4.13	0.27	24.09
1936	11.16	1.38	2.94	0.42	2.50	6.26	0.19	2.28	15.65	3.35	46.14
1937	13.59	2.06	6.98	0.51	4.32	7.82	0.19	2.45	24.91	4.64	67.47

Data for 1920 to 1933 compiled by National Association of Purchasing Agents[39] from records of the Bureau of Dairy Industries, U. S. Department of Agriculture. Data for 1936 and 1937 from Bureau of Agricultural Economics.

In 1937 the other states reporting production of casein and the amounts produced were as follows: Arizona 0.16, Arkansas 0.004, Indiana 0.10, Iowa 0.49, Maine 0.19, Missouri 0.75, Montana 0.10 Ohio 0.47, Oklahoma 0.02, Oregon 0.36, Tennessee 0.002, Texas 0.07, Utah 0.21, Washington 1.63 Wyoming 0.08. Colorado and Kansas reported small production in 1936 but none in 1937.

1929, and 87.5 per cent in 1935, if the total consumption indicated in Table 32 is correct. For a great many years it has been commonly estimated that 75 to 80 per cent of the casein consumed goes into paper coating[14, 39, 40] (see also first edition of this book, p. 259) but it is difficult to reconcile so high an estimate with consumption claimed in glue, plastics, paints, and insecticides. It is particularly difficult to understand a decrease in percentage of total consumption for purposes other than paper coating between 1929 and 1935 because casein plastics and casein paints made striking gains during that period. According to the Census data, paper and glue in 1929 accounted for 43.6 out of 58.1 million pounds of casein consumed, leaving a balance of 5.5 million pounds for plastics, paints, and all other uses; while in 1935 paper and glue accounted for 41.6 million pounds although the total consumption according to Table 32 was only 40.9 million pounds and both plastics and paints used much more casein than in 1929.

The Census statistics for the paper industry do not report coated papers as a separate item. Most coated paper is included in the items for book and fine papers. The reported consumption of casein in paper coating in 1929 amounts to 17.9 pounds per ton of such papers. Table 34 is prepared on the assumption, not necessarily true, that this factor has been reasonably constant and may be applied to the data for production of book and fine papers from 1919 to 1935 to estimate the

TABLE 34.—An Estimate of the Consumption of Casein in Paper Coating and Its Percentage of Total Consumption of Casein, Based on the Production of Book and Fine Papers from 1919 to 1935

Year	Production of book and fine paper[a]	Casein consumed in coating paper[b]	Total consumption of casein[c]	Percentage of total consumption used in paper
	million tons	*million pounds*	*million pounds*	
1919	1.144	20.5	22.53	91
1922	1.324	23.7	21.27	111
1925	1.837	32.9	35.46	93
1926	1.903	34.1	42.23	81
1927	1.767	31.6	42.24	75
1928	1.859	33.3	50.88	65
1929	2.064	36.9[a]	58.12	63.5
1930	1.934	34.6	60.46	57
1931	1.675	30.0	38.84	77
1932	1.353	24.2	25.90	93
1933	1.541	27.6	32.41	85
1935	1.789	35.7[a]	40.87	87.5

[a] Data reported by the Bureau of the Census.
[b] Calculated on the assumption that 17.9 pounds of casein are consumed for each ton of book and fine paper produced, which was the case in 1929. For 1929 and 1935 the data are reported by the Census.
[c] From Table 32.

consumption of casein in paper coating and its percentage of the total consumption of casein given in Table 32.

The calculations of Table 34 emphasize the unreasonable apparent shrinkage in consumption of casein by industries other than paper making since 1930, a time when at least two of them were greatly expanding their consumption. Moreover, for the years between 1919 and 1925 the difference between the total consumption and consumption in paper coating is much less than the estimates of consumption of casein in glue from several well-informed sources. For example, in 1922, when the calculation of the casein used in paper coating materially exceeds the estimated total consumption, the amount of casein used in casein glue was believed to be between 4.5[18] and 7.0 million pounds.[40]

In explanation of these discrepancies it may be pointed out that the Biennial Census of Manufactures reports larger domestic production of casein than does the Bureau of Agricultural Economics. Data of the two Bureaus are compared in Table 35. In part the difference between them lies in the fact that the Bureau of Agricultural Economics reports only "dried casein manufactured in factories." Small producers of casein, however, sometimes dispose of it in the form of wet curd. When the wet curd is sold to larger manufacturers or dealers in casein it presumably appears in the statistics of domestic production of dried casein,

but when it is sold directly to consumers it probably does not. Some consumers of casein located in regions of casein production find it economical to buy wet casein locally and dry and blend it for their own use. Wet casein sold directly to consumers would not appear in the reports of the Bureau of Agricultural Economics but it does appear in the Biennial Census, while wet casein sold to larger dealers in casein perhaps involves some duplication in the reports of the Biennial Census. At any rate, the trends revealed in the last column of Table 34 may mean that in the prosperous years from 1919 to 1929 producers of wet curd were increasingly content to sell it to the larger dealers, whereas the business depression following 1929 and 1930 marked a revival of the old procedure of selling wet curd directly to consumers.

TABLE 35.—Domestic Production of Casein as Reported by the Bureau of Agricultural Economics and by the Biennial Census of Manufactures

Year	Bureau of Agricultural Economics dry casein only	Biennial census of manufactures dry casein	wet casein	dry and wet casein
	pounds	*pounds*	*pounds*	*pounds*
1925	16,660,000			21,977,789
1927	18,033,000			23,516,481
1929	30,537,000			57,826,167
1931	35,335,000			41,375,639
1933	24,087,000	30,119,739	1,403,894	
1935	37,638,000	43,276,254	5,700,715	

Since adequate information about the consumption of casein by various industries is not available, a plausible guess may be of interest. For 1937 the following is suggested:

Paper coating	52 million pounds, or	71.2 per cent
Plastics	8 " "	11.0 " "
Glue	7 " "	9.6 " "
Paint	3 " "	4.1 " "
Insecticide sprays	1 " "	1.4 " "
All other uses	2 " "	2.7 " "
	73 " "	100.0 " "

The writer is inclined to believe that this guess overestimates the proportional consumption in paper coating and underestimates the consumption by "all other uses."

For comparison with the above guess about consumption in the United States, statistics are available for the consumption of casein in Germany. In 1937 Germany[47] used 18,000 metric tons or 39.7 million pounds, which is 0.61 pound per capita, practically the same as the probable per capita consumption in the United States. In 1932 the consumption in Germany[11] was divided among industries as follows:

Paper coating......	8.38	million pounds,	or	26.7	per	cent
Glue for plywood...	7.72	"	"	24.6	"	"
Plastics...........	6.61	"	"	21.1	"	"
Edible casein......	4.85	"	"	15.5	"	"
Paint and glue.....	2.65	"	"	8.5	"	"
Sizing, tanning, soap and medicinal....	1.10	"	"	3.6	"	"
Total.........	31.31	"	"	100.0	"	"

The German supply of casein is said to be less than the requirements, so that the present effort is to replace casein wherever possible with other materials produced in that country.[26, 47]

References

1. Andrew, R. E. and Garman, P., *J. Econ. Entomol.*, **19**, 671 (1926); *Conn. Agr. Expt. Sta. Bull.* **278**, 491 (1926)
2. Anonymous, *Parfumerie Moderne* (Sept. 1918)
3. Anonymous, *World's Paper Trade Rev.*, **46**, 281, 657
4. Anonymous, *Kunstdunger und Leim,* **1931**, 487; *Oil Color Trades J.*, **81**, 971 (1932)
5. Anonymous, *Farbe und Lack,* **1937**, 426
6. Bennett, H., "The Chemical Formulary," New York, D. van Nostrand Co., Inc., 1933-1936
7. Biddle, A., British Pat. 253,740 (June 24, 1926)
8. Blum, W., "Addition agents in electrodeposition," Ch. 33 in J. Alexander, "Colloid Chemistry," New York, Reinhold Publishing Corp., 1932
9. Boult, J., British Pat. 19,297 (Sept. 14, 1908)
10. British Pat. 12,887 (1905)
11. Bureau of Foreign and Domestic Commerce, U. S. Dept. of Commerce, "World Trade Notes on Chemicals and Allied Products," p. 11, Nov. 2, 1935
12. Carbide and Carbon Chemicals Corp., "Emulsions" (technical booklet), p. 51, Oct. 1936
13. Carbide and Carbon Chemicals Corp., *Ind. Eng. Chem., News Ed.*, (*adv.*), Feb. 20, 1938
14. Chappell, F. L., *Food Ind.*, **3**, 102 (1931)
15. Department of Agriculture (U. S.), *Statistical Bulletin No. 25*
16. Ewe, G. E., *Pract. Drug.*, **41**, No. 3, 22 (1923)
17. Fischer, C. H., *Deut. Textilwirt.*, **4**, No. 21, 24 (1937)
18. Forest Products Laboratory, private communication
19. French Pat. 151,014
20. Gateau, J. T., British Pat. 20,740 (June 18, 1908)
21. Genin, G., *Lait,* **16**, 1083 (1936)
22. German Pat. 202,108 (May 15, 1907)
23. Glyco Products Co., Inc., "Trigamine" (folder) 1936
24. Harry, R. G., *Paint Manuf.*, **7**, No. 7, 214 (1937)
25. Haskell, W. E., *Concrete,* **28**, No. 6, 18 (1926)
26. Hoyer, F., *Gelatine Leim Klebstoffe,* **3**, 174 (1935)
27. I. G. Farbenindustrie A.-G., British Pat. 373,233; Abridg. Spec., 1932, Group IV, 312
28. Kirschbraun, L., U. S. Pat. 1,275,043 (May 27, 1918)
29. Kline, G. M., *Ind. Eng. Chem.*, **27**, 556 (1935)
30. Knight, G. D., U. S. Pat. 1,816,978 (Aug. 4, 1931)

31. Kühl, H., *Südd. Apoth. Ztg.*, **52**, 390
32. Kühl, H., *Seifensieder-Ztg.*, **51**, 551 (1924)
33. Laeufer, J., U. S. Pat. 1,724,718 (Aug. 13, 1929)
34. Lovett, A. L., *Oregon Agric. College Expt. Sta. Bull.*, **169** (1920)
35. Lynch, F. C. C., *Can. Chem. Met.*, **9**, 203, 221, 264 (1925); **10**, 13 (1926)
36. Marsek, F. A., *Am. Perfumer*, **14**, 367 (1919); **15**, 87 (1920)
37. Mayer, H. T., *Farben-Chem.*, **6**, 24 (1935)
38. McGivern, W. L., J. H. Foster Co., and Swift, R., British Pat. 251,098 (Apr. 29, 1926)
39. National Assoc. of Purchasing Agents, Report A-15, "Casein," Sept. 1935; data compiled partly from *Oil Paint & Drug Reporter*, Nov. 7 and 14, 1934
40. National Resources Intelligence Service, Dept. of the Interior, Ottawa, Canada, "Casein and Its Industrial Applications" (about 1924)
41. Ohl, F., *Gelatine Leim Klebstoffe*, **3**, 169 (1935)
42. Pincass, H., *Seide*, **37**, 238, 240 (1932)
43. Rasser, E. O., *Kunstoffe*, **19**, 6 (1929)
44. Richardson, *J. Soc. Dyers and Colorists*, **25**, 48 (1919)
45. Robinson, *J. Agric. Research*, **31**, 71 (1925)
46. Ryan, J. F., U. S. Pat. 1,132,687 (Mar. 23, 1915)
47. Sauer, E. and Hagenmüller, K., *Kolloid-Z.*, **83**, 210 (1938)
48. Schlitt, W., *Gelatine Leim Klebstoffe*, **1**, 147 (1933)
49. Shirley, C. F., representing the United Typothetae of America and 75 manufacturers of coated paper, in a statement to the joint Congressional Committee on Tariffs in hearings on House Resolution 7456 (Sept. 1922)
50. Smith, R. H., *J. Econ. Entomol.*, **26**, 730 (1933)
51. Soc. Veuve P. Chouanard & Fils Co., French Pat. 788,406
52. Stocks, H. B., First Report on Colloid Chemistry (British), Dept. of Scientific and Industrial Research, p. 69 (1917) and Clayton, W., Second Report, p. 100 (1921)
53. Teague, M. C. and Brewster, N. H., U. S. Pat. 1,957,301 (May 1, 1934)
54. Trucco, A., *Industria chimica*, **8**, 826 (1933)
55. Tsubata, M., British Pat. 224,273 (Oct. 30, 1924)
56. U. S. Patents 1,621,541, 1,621,542, 1,621,543, 1,724,603, cited by National Assoc. Purchas. Agents, Ref. 39.

56a. Van Allen, L. R., U. S. Pat. 2,134,898 (Nov. 1, 1938)

57. Volck, W. H., U. S. Pat. 1,707,466 (Apr. 2, 1929)

Author Index

Italic numerals have been used to indicate those pages upon which an author's name appears. The citation number of any author's reference can be obtained from the bibliography by referring to the page indicated by italics, and using this number in referring to the pages otherwise indicated.

Subject Index

MEMORANDA

MEMORANDA

MEMORANDA

AMERICAN CHEMICAL SOCIETY MONOGRAPH SERIES

PUBLISHED

No.
26. The Chemistry of Wheat Flour
By C. H. Bailey.
27. Surface Equilibria of Biological and Organic Colloids
By P. Lecomte du Noüy.
28. The Chemistry of Wood
By L. F. Hawley and Louis E. Wise.
29. Photosynthesis
By H. A. Spoehr. (Out of Print)
31. Equilibria in Saturated Salt Solutions
By Walter C. Blasdale.
32. Statistical Mechanics as Applied to Physics and Chemistry
By Richard C. Tolman. (Revision in preparation)
33. Titanium
By William M. Thornton, Jr.
34. Phosphoric Acid, Phosphates and Phosphatic Fertilizers
By W. H. Waggaman.
35. Noxious Gases
By Yandell Henderson and H. W. Haggard. (Out of Print)
36. Hydrochloric Acid and Sodium Sulfate
By N. A. Laury.
37. The Properties of Silica
By Robert B. Sosman.
38. The Chemistry of Water and Sewage Treatment
By Arthur M. Buswell. (Revision in preparation)
39. The Mechanism of Homogeneous Organic Reactions
By Francis O. Rice.
40. Protective Metallic Coatings
By Henry S. Rawdon. To be revised by H. M. Burns.
41. Fundamentals of Dairy Science (Revised Edition)
By Associates of Rogers.
42. The Modern Calorimeter
By Walter P. White.
43. Photochemical Processes
By George B. Kistiakowsky.
44. Glycerol and the Glycols
By James W. Lawrie.
45. Molecular Rearrangements
By C. W. Porter.
46. Soluble Silicates in Industry
By James G. Vail.
47. Thyroxine
By E. C. Kendall.
48. The Biochemistry of the Amino Acids
By H. H. Mitchell and T. S. Hamilton. (Revision in preparation.)
49. The Industrial Development of Searles Lake Brines
By John E. Teeple.
50. The Pyrolysis of Carbon Compounds
By Charles D. Hurd.
51. Tin
By Charles L. Mantell.
52. Diatomaceous Earth
By Robert Calvert.
53. Bearing Metals and Bearings
By William M. Corse.
54. Development of Physiological Chemistry in the United States
By Russell H. Chittenden.
55. Dielectric Constants and Molecular Structure
By Charles P. Smyth.

AMERICAN CHEMICAL SOCIETY MONOGRAPH SERIES

PUBLISHED

No.

56. **Nucleic Acids**
    By P. A. Levene and L. W. Bass.
57. **The Kinetics of Homogeneous Gas Reactions**
    By Louis S. Kassel.
58. **Vegetable Fats and Oils**
    By George S. Jamieson.
59. **Fixed Nitrogen**
    By Harry A. Curtis.
60. **The Free Energies of Some Organic Compounds**
    By G. S. Parks and H. M. Huffman.
61. **The Catalytic Oxidation of Organic Compounds in the Vapor Phase**
    By L. F. Marek and Dorothy A. Hahn.
62. **Physiological Effects of Radiant Energy**
    By H. Laurens.
63. **Chemical Refining of Petroleum**
    By Kalichevsky and B. A. Stagner. (Out of Print)
64. **Therapeutic Agents of the Quinoline Group**
    By W. F. Von Oettingen.
65. **Manufacture of Soda**
    By T. P. Hou.
66. **Electrokinetic Phenomena and Their Application to Biology and Medicine**
    By H. A. Abramson.
67. **Arsenical and Argentiferous Copper**
    By J. L. Gregg.
68. **Nitrogen System of Compounds**
    By E. C. Franklin.
69. **Sulfuric Acid Manufacture**
    By Andrew M. Fairlie.
70. **The Chemistry of Natural Products Related to Phenanthrene (Second Edition with Appendix)**
    By L. F. Fieser.
71. **The Corrosion Resistance of Metals and Alloys**
    By Robert J. McKay and Robert Worthington.
72. **Carbon Dioxide**
    By Elton L. Quinn and Charles L. Jones.
73. **The Reactions of Pure Hydrocarbons**
    By Gustav Egloff.
74. **Chemistry and Technology of Rubber**
    By C. C. Davis and J. T. Blake.
75. **Polymerization**
    By R. E. Burk, A. J. Weith, H. E. Thompson and I. Williams.
76. **Modern Methods of Refining Lubricating Oils**
    By V. A. Kalichevsky.
77. **Properties of Glass**
    By George W. Morey.

IN PREPARATION

Piezo-Chemistry
By L. H. Adams.

The Chemistry of Coordinate Compounds
By J. C. Bailar.

Water Softening
By A. S. Behrman.

The Biochemistry of the Fats and Related Substances
By W. R. Bloor.

Ions, Dipole Ions and Uncharged Molecules
By Edwin J. Cohn.

Carbohydrate Metabolism
By C. F. Cori and G. T. Cori.

Properties of Water
By Dr. N. E. Dorsey.

Organometallic Compounds
By Henry Gilman.

Surface Energy and Colloidal Systems
By W. D. Harkins and T. F. Young.

Raman Spectra
By James H. Hibben.

Raw Materials of Lacquer Manufacture
By J. S. Long.

Significance of Manganese, Iron and Aluminum to Soil Acidity and Plant Life
By Forman T. McLean.

Phenomena at the Temperature of Liquid Helium
By E. F. Burton.

Acetylene
By associates of the late J. A. Nieuwland.

Photochemical Reactions in Gases
By W. A. Noyes, Jr., and P. A. Leighton.

Furfural and other Furan Compounds
By F. N. Peters, Jr., and H. J. Brownlee.

Aliphatic Sulfur Compounds
By E. Emmet Reid.

Electrical Precipitation of Suspended Particles from Gases
By W. A. Schmidt and Evald Anderson.

Mineral Metabolism
By A. T. Shohl.

Aluminum Chloride
By C. A. Thomas.

Precise Electric Thermometry
By W. P. White and E. F. Mueller.

Measurement of Particle Size and Its Application
By L. T. Work.